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Ethanol Production by Enzymatic Hydrolysis

Parametric Analysis of a Base-Case Process

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NOTICE

PREFACE

The Solar Fuels Research Division of the Solar Energy Research Institute is responsible for managing a program of research and development for the U.S. Department of Energy Office of Alcohol Fuels. One emphasis of the program is the hydrolysis of lignocellulosic feedstock to sugars and the subsequent fermentation of these sugars to fuel ethanol. One of the more promising means to accomplish this is with an enzymatic process. The specificity and relatively mild conditions of the enzymatic reaction provide the potential for complete utilization of cellulose and inexpensive materials of construction for process equipment. This report presents an analysis of one enzymatic hydrolysis process in which the unit operations of enzyme production, hydrolysis of cellulose to glucose, and fermentation of the glucose to ethanol are performed separately.

This report contains a process flow sheet and an economic analysis of a base-case design. Background information on the technology of enzyme hydrolysis and on the bases for the base-case design assumptions is included. A parametric analysis of the base-case design is provided to identify key research issues and determine the ultimate potential of this process.

This study was carried out by Steven Isaacs of the Alcohol Fuels Program Office with assistance and guidance from Larry Douglas, Bill Hoagland, John Wright, and Karel Grohmann.

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SUMMARY

Objective

To present a base-case flowsheet for an enzymatic hydrolysis process, to perform a parametric sensitivity analysis to identify key research issues, and to assess the potential of this technology. Background information concerning the enzyme hydrolysis technology is included.

Discussion

The plant discussed in the report is a large-scale facility, producing 50 million gallons of ethanol per year. The plant design is based on the process originally conceived by the U.S. National Army Command and consists of these process steps: pretreatment; enzyme production; enzyme hydrolysis; fermentation; and distillation. The base-case design parameters are based on recent laboratory data from Lawrence Berkeley Laboratories and the University of California at Berkeley. The selling price of ethanol is used to compare variations in the base-case operating parameters, which include hydrolysis efficiencies, capital costs, enzyme production efficiencies, and enzyme recycle.

Conclusion

The economic evaluation of the base-case process design indicates a selling price of $2.13/gal. Major contributions to this cost include the aspen wood feedstock cost (46.3¢/gal), power costs (14.3¢/gal), and capital-related costs. The capital costs are dominated by the steam explosion and enzyme production sections (35.4% and 42.6% of the total onsite purchased capital).

The most significant improvement to the base-case process economies is caused by the inclusion of enzyme recycle. Sixty percent recycle via adsorption onto freshly pretreated cellulosics decreases the selling price to $1.67/gal. Ability to ferment xylose, a five-carbon sugar derived from the hydrolysis of the hemicellulose functions of biomass, reduces the selling price to $1.70/gal with no enzyme recycle and $1.38 with 60% enzyme recycle.

Other process parameters that have significant impact on ethanol selling price include hydrolysis conversion, enzyme loading per gram of cellulose, and reduction in the capital cost associated with the equipment for enzyme production and steam explosion pretreatment. Combinations of improvements in these parameters cause a reduction in the base-case selling price to 99.0¢/gal.

Results of the parametric analyses indicate potential for this particular enzymatic hydrolysis process to become competitive with the current market price for ethanol and for ethanol produced from biomass via an acid hydrolysis process. Uncertainties in the scale up of laboratory results, uncertainties in actual industrial scale agitation, aeration and sterility requirements, and lack of detail in the computer simulation model make the selling prices quoted

in this report only approximate. Additional research at both the laboratory scale and the larger scale is required for a more accurate design and cost estimation.

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SECTION 1.0

INTRODUCTION

Current research at SERI includes the development of processes that produce ethanol from cellulose for use as a fuel. Ethanol may be used as an octane enhancer, as a fuel extender when blended with unleaded gasoline, or as a neat fuel. Cellulose is attractive as a raw material because it is abundant and renewable; an estimated 609 billion metric tons of biomass (wood, crop residues, and municipal waste) are produced each year in the United States alone (SRI International 1981).

Currently most ethanol intended for industrial use is produced from ethylene, a petroleum product. Beverage ethanol is produced by fermentation of starch- and sugar-rich feedstocks. Use of cellulose will provide a renewable feedstock for industrial ethanol, yet will not compete with the supply of food.

Cellulose may be converted to ethanol in two discrete steps. The cellulose is hydrolyzed to glucose, which is fermentated to ethanol. Fermentation technology has been in development for years by the beverage industry. Therefore, more recent research efforts have concentrated on the development of economical means of carrying out the hydrolysis step.

Cellulose may be hydrolyzed to glucose chemically with acid or enzymatically. Acid hydrolysis processes have the disadvantage that they require severe conditions of high temperature and low pH. Such conditions make it necessary to employ expensive corrosive-resistant equipment. Conversion efficiency is limited due to glucose degradation. Enzymatic processes have been severely hampered by the high costs associated with enzyme production, the limited accessibility of the substrate to the enzyme, and slow reaction rates. However, enzymatic processes are performed at less severe reaction conditions and have the potential, due to the high specificity of enzymatic reactions, for total conversion of cellulose to glucose.

Many flowsheets for the enzymatic cellulose-to-ethanol process have been proposed and all fit into one of three categories: (a) separate hydrolysis, fermentation, and enzyme production steps; (b) simultaneous saccharification and fermentation (SSF), which combines the hydrolysis and fermentation reactions into one step; and (c) direct microbial conversion, which combines all three steps of enzyme production, hydrolysis, and fermentation. The objective of this work is to evaluate an enzymatic hydrolysis process that fits into the first category because this type of process is furthest along in development toward operation at a scale larger than bench scale. A base-case flowsheet is presented based on the model developed by Chem Systems Inc., under a SERI subcontract, and on recent laboratory results obtained at Lawrence Berkeley Laboratories. Using this simulation model, a parametric analysis is performed to assess the economic feasibility of such a process and to determine key research areas for future process improvement.

SECTION 2.0

OBJECTIVE AND SCOPE

The objective of this report is to present the economics of a base-case enzymatic hydrolysis cellulose-to-ethanol plant, and to describe the effect of variations in process parameters on the selling price of ethanol.

The base-case process consists of steam-exploded pretreatment, enzyme production by fed-batch fermentation of the RUT C30 strain of the fungus Trichoderma viride, hydrolysis of cellulose to glucose followed by glucose fermentation in a separate process step, and vapor reuse distillation. A detailed process description is presented in Section 4.0.

In the parametric analysis, changes are considered in operating conditions (e.g., enzyme to cellulose ratio); anticipated operational results (e.g., cellulose conversion); technology in the form of breakthroughs (e.g., xylose fermentation by yeast); and anticipated equipment costs (e.g., for the enzyme production and steam explosion sections). The only major change in process configuration is the addition of an enzyme recycle section.

The purpose of the parametric analysis in this report is to assess the economic feasibility of this particular enzymatic process at various levels of implementation of process improvements, as well as to determine the areas of fundamental and process research that would produce significant cost reduction. This report does not provide a comparison of the various enzymatic hydrolysis processes, and only a brief discussion of alternative processes (e.g., simultaneous saccharification and fermentation [SSF] and direct conversion) is included in Appendix A.

It is also beyond the scope of this report to produce a detailed process design and an exact cost for ethanol production. The simulation program models the back end of the process (fermentation, distillation, waste treatment, and heat generation) with little detail. Emphasis is placed on the enzymatic portion of the plant (pretreatment, enzyme production, and hydrolysis). A comprehensive kinetic model to describe cellulose conversion for various pretreatments, feedstocks, and hydrolysis conditions is not currently available, and the parametric analysis does not account for these interactions. Finally, industrial-scale equipment costs and operating conditions for some of the processes are speculative, particularly those of steam explosion and enzyme production.

SECTION 3.0

ENZYMATIC HYDROLYSIS

This section presents a brief overview of the fundamentals of enzymatic hydrolysis of biomass to provide background information for the process design and parametric analysis of this report.

3.1 FEEDSTOCK CHARACTERISTICS

Cellulose does not occur in nature in a pure form. It is always found associated with other materials necessary for biomass structure and growth. Typical compositions of corn stover and aspen wood are shown in Table 3-1.

Aspen wood has a higher water content than field-dried corn stover (50 wt % and 30.7 wt %, respectively). On a dry basis, aspen wood has a higher cellulose content than corn stover. For the example of Table 3-1, the cellulose contents are 38 wt % for corn stover and 49 wt % for aspen wood. The dry-basis lignin content of the aspen wood (17 wt %) is higher than that of the corn stover (10 wt %), but both exhibit similar dry-basis hemicellulose contents. The ash content of corn stover is significantly higher than that of aspen wood.

Table 3-1. Feedstock Composition

Component	Wet Basis		Dry Basis	
	Field Dry Corn Stover (wt %)	Aspen Wood (wt %)	Field Dry Corn Stover (wt %)	Aspen Wood (wt %)
Water	30.7	50.0	0.0	0.0
Crystalline cellulose	22.5	20.7	32.5	41.4
Amorphous cellulose	3.9	3.7	5.6	7.4
Hemicellulose				
Pentosan	16.1	10.9	23.2	21.8
Hexosan	6.5	4.4	9.4	8.8
Carbohydrates	5.3	0.0	7.6	0.0
Insoluble lignin	7.0	8.3	10.1	16.6
Insoluble protein	3.0	0.0	4.3	0.0
Ash	3.9	0.1	5.6	0.2
Extractives	1.1	1.9	1.6	3.8
Total	100.0	100.0	100.0	100.0

Figure 3-1 shows a schematic representation of wood. Lignins and hemicelluloses are located primarily in the middle lamella, forming a protective adhesive shell for the cells. Cellulose is located in the primary and secondary cell walls. Many texts provide a more detailed description of wood chemistry, one of which is Wenzl (1970).

Cellulose is a linear polymer of D-glucose molecules (a six-carbon sugar) held together by β-1,4-glycosidic bonds (Figure 3-2). The average chain length is highly dependent on the type of biomass and pretreatment, but analyses reveal typical degrees of polymerization between 700 and 2000 units (Wenzl 1970). The structure of cellulose in plant tissue has been shown to exist in crystalline and amorphous forms. Cellulose chains aggregate in parallel fashion, bound together by hydrogen bonding between hydroxyl groups, to form crystalline regions of high mechanical strength and high chemical and enzymatic stability. Primary chains may be up to 30 times the length of the region of crystallinity and may emerge from either end in a more tangled fashion, forming less ordered, less stable amorphous regions (Burr 1947).

Hemicellulose in crop residues and hardwoods is a complex polysaccharide composed mainly of D-xylose (a five-carbon sugar), and D-glucose, L-arabinose, and organic acids. Hemicellulose is less crystalline than cellulose and therefore can be readily hydrolyzed in dilute alkali and warm dilute acid solution.

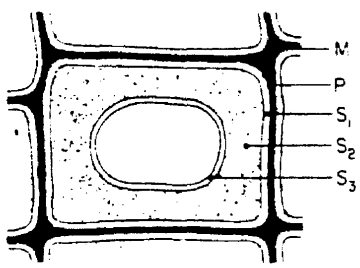


Figure 3-1a. Cross Section of a Wood Fiber Cell

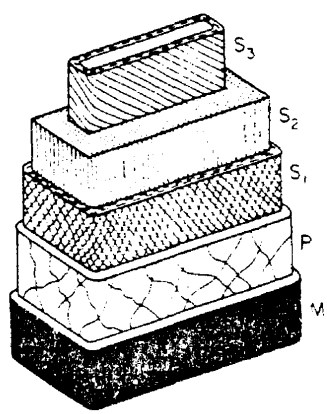


Figure 3-1b. Model of a Conifer Tracheid. M = middle lamella; P = primary wall; S_1 = outer layer of the secondary wall; S_2 = secondary wall; S_3 = inner layer of the secondary wall (also called the tertiary wall)

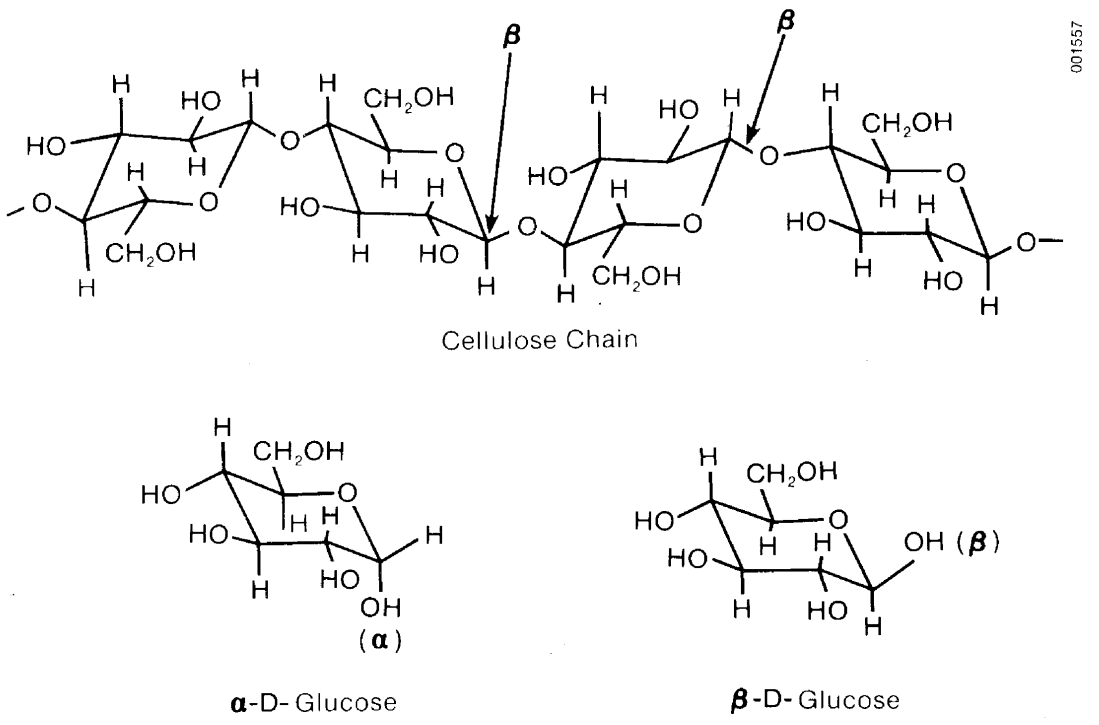


Figure 3-2. Chemical Structure of a Cellulose Chain, α-D-glucose, and β-D-glucose

Carbohydrate (starch) is a polymer of glucose held together by α-glycosidic bonds. Starch consists of both linear polymers (amylose) with a degree of polymerization of 60-300 and branched polymers (amylopectin) with a degree of polymerization of 300-6000. The tertiary structures of both forms, similar to that of hemicellulose, do not allow for close packing to form a stable crystalline form, and therefore the starch is amorphous and readily hydrolyzed.

Lignin is a heterogeneous, amorphous, branched polymer based primarily on the phenylpropane unit. It is intermixed with the hemicellulose in a layer surrounding cellulose. The exact structure of lignin is not known.

In addition to cellulose, hemicellulose, carbohydrates, and lignins, plant matter is composed of a variety of other materials, including ash and extractives. Due to the high variability of these materials with species and seasons, only a limited amount of information is available on them.

3.2 CELLULASE ENZYMES

Enzymatic hydrolysis of cellulose to ethanol is performed by a class of enzymes termed cellulases. Each component of the cellulase multienzyme system plays a particular catalytic role in the depolymerization of cellulose to glucose monomer units.

Sources of cellulase enzymes include anaerobic protozoa, aerobic fungi, and aerobic and anaerobic bacteria. The most extensively studied cellulase enzymes have been produced from aerobic, mesophilic fungi, and of these,

current research is focused primarily on strains of Trichoderma reesei. These fungi are of particular interest because they produce, extracellularly, all the enzyme components necessary for cellulose hydrolysis; the cellulase produced is resistant to chemical inhibitors and stable at temperatures up to 50°C for up to 48 hours (Mandels 1980).

Figure 3-3 presents the genealogy of mutational research of T. reesei strains (Wilke 1981). The wild type strain, QM6A, was isolated by Reese in 1950 (Reese 1950). Many hydrolysis studies have been performed with the QM9414 mutant isolated at Rutgers, but the extracellular cellulase from this strain is deficient in the enzyme necessary for the cleavage of cellobiose to glucose (β-glucosidase). The RUT C30 strain, also isolated at Rutgers (Montenecourt 1977), overcomes this obstacle since it displays high β-glucosidase activity. The RUT C30 strain is also of interest because it is resistant to catabolite repression, is hyper-cellulase producing, and exhibits high xylanase activity.

Hyper-cellulase production signifies that the strain produces the cellulase in abundance, perhaps producing greater amounts than required for culture growth. Catabolite repression, a form of metabolic control, occurs where the production of an enzyme is stopped due to the presence of a breakdown product of the reaction catalyzed by that enzyme. Resistance to catabolite repression implies that the RUT C30 strain will continue to produce enzymes even after

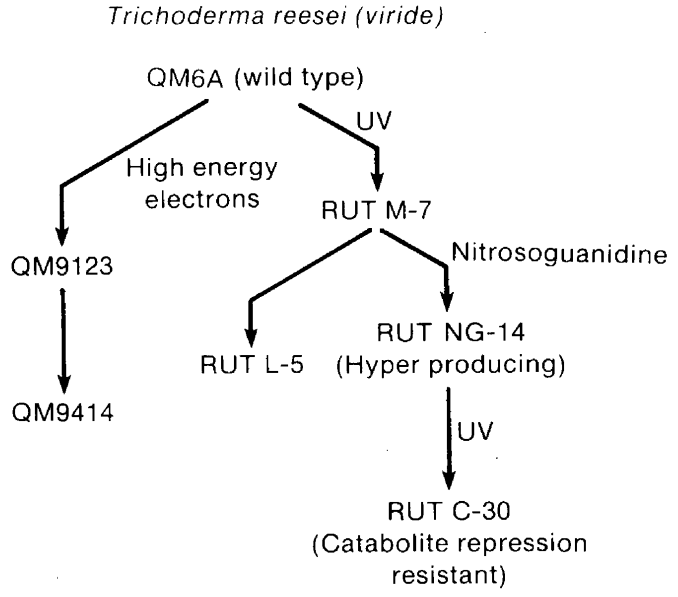


Figure 3-3. Genealogy of Mutants of Trichoderma reesei (formerly Trichoderma viride) That Produce High Yields of Cellulase

significant amounts of cellulose, present for cell growth and enzyme induction, have been degraded. Xylanase activity, which is a term for the enzyme activity for the hydrolysis of xylans to xylose or short-chain polymers of xylose, would be beneficial to a biomass-to-ethanol process both in the utilization of the five-carbon sugars, either through fermentation to ethanol or the production of other salable by-products, and for removal of the protective coating that the xylans may provide against the enzymatic degradation of cellulose.

Several theories have been proposed for the exact mode of action of cellulases. Originally, Reese and co-workers proposed the C_1-C_x concept (Reese 1950). This was a mechanism in which two types of enzymes, C_1 and C_x, acted sequentially to depolymerize the cellulase chain. It was thought that the C_1 component converted the cellulose to a reactive state that was subsequently depolymerized by the C_x component.

More recent fractionation studies suggest another mechanism of cellulase action. The cellulase complex has been shown to consist of three main types of enzymes: β-1,4-glucan glucanohydrolase, an endo-enzyme; β-1,4-glucan cellobiohydrolase, an exo-enzyme; and β-glucosidase.

The first enzyme, β-1,4-glucan glucanohydrolase, randomly acts on the interior of the cellulose polymer to generate new chain ends. The second enzyme, β-1,4-glucan cellobiohydrolase, catalyzes the cleavage of a cellobiose unit from the nonreducing end of the cellulose chain. It appears to be end product inhibited and required for the hydrolysis of highly ordered substrates.

The last major component of cellulase, β-glucosidase, acts to split cellobiose and other short-chain polymers of glucose to the monomeric glucose unit. This component has been found to be end product inhibited, acting by a noncompetitive mechanism.

Figure 3-4 illustrates the separate activities of the cellulase complex (from Wilke 1981). The C_x enzyme activity from the original postulate of cellulase mechanism is considered equivalent to the random-acting endoglucanase from the current theory. Likewise, the old C_1 activity is considered equivalent to the current exoglucanase activity. Both enzymes have been found to act synergistically; that is, fractionation studies have indicated that the combined enzymes have a greater activity against cellulose than the sum of each component.

3.3 LIMITS ON CELLULOSE HYDROLYSIS

In theory, due to the specificity of the enzymatic reaction, cellulose can be converted to glucose with 100% efficiency. However, in application, yields of glucose will be bounded by factors that influence the rate and extent of cellulose hydrolysis.

The hydrolysis rate will depend on cellulose accessibility and crystallinity. Surface area available for enzyme-substrate interaction will be influenced by pore size and shielding effects by lignins. The crystalline structure excludes water molecules as well as any larger molecules and thus

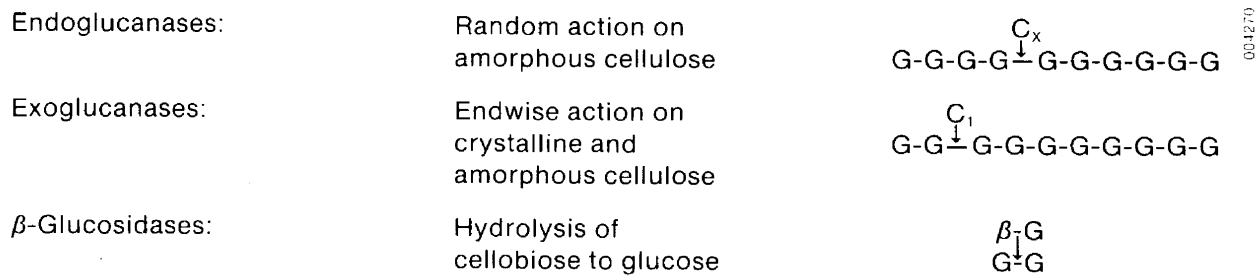


Figure 3-4. Separate Activities of the Cellulase Complex

reduces available surface area. Crystalline regions will be hydrolyzed at a much slower rate than amorphous cellulose due to the greater stability imparted by interchain hydrogen bonding.

In the case of a high-cost biomass substrate, a more important factor than rate of reaction may be the extent of reaction. Steric effects imparted by crystalline structure may effectively halt reaction. More importantly, shielding effects due to lignins may place a limit on cellulose available for reaction.

Product inhibition may limit reaction rate and extent. Glucose has been shown to inhibit β-glucosidase, and cellobiose has been shown to inhibit the cellulose-hydrolyzing enzymes.

3.4 PRETREATMENT

The primary aim of pretreatment is to enhance the enzymatic susceptibility of native biomass, through chemical or physical means. Physical pretreatments include various forms of milling, shredding, and mulching. All act to decrease the particle size and increase surface area as well as decrease the degree of polymerization and crystallinity. Ball and roll milling are the two major techniques. An improvement over ball milling appears to be simultaneous wet milling and enzymatic hydrolysis (Kelsey 1980). The high energy requirements make the economic feasibility of industrial-scale operation of these physical pretreatments questionable.

Chemical pretreatments enhance enzymatic susceptibility by removing the shielding effects of lignin, reducing crystallinity, and increasing cellulose solubility by addition of chemical substitutes or hydration to swell cellulosis fibers. A SERI report (Chum 1983) discusses the relative costs of some of these pretreatments.

Feedstock costs, which are predicted to be $30/dry ton, will play a major role in the production cost of ethanol. For example, at 100% cellulose conversion to glucose and 90% efficiency in fermentation of glucose to ethanol, and assuming 50% cellulose content on a dry basis, costs associated with the feedstock are about 37¢/gal. At only 50% cellulose conversion, these costs become about 74¢/gal. Due to the importance of enhanced biomass utilization, pretreatment effectiveness must be considered as well as pretreatment cost.

Recent laboratory results indicate that three methods of chemical pretreatment may prove to yield high enzymatic conversions and be cost effective. Organosolv methods currently suffer from the high costs of solvent recovery, but separations research may produce an economical process. The Dartmouth acid pretreatment exhibits high conversions, but currently suffers from the acid costs and the need for a milled substrate. Steam explosion may not require milling, but has the disadvantage of high steam requirements, costly equipment, and is a batch, rather than continuous, process.

Lignin removal prior to cellulose hydrolysis may prove beneficial due to (a) the possibility of a toxic effect of lignins on yeasts; (b) the possibility that some cellulase components may bind to lignins, reducing efficiency and limiting enzyme recovery and reuse; (c) reduction of inert solids in the hydrolysis section; and (d) separation of lignins for sale as a chemical by-product. Pretreatments such as organosolv or alkali wash will separate lignins from cellulose.

3.5 ENZYMATIC ACTIVITY MEASUREMENT

Cellulose activity is reported in terms of filter paper units (FPU) in this study. A brief discussion of cellulase activity measurements is included here.

The activity of an enzyme is usually defined in terms of the amount of product formation per unit of time. An exact determination of cellulase activity presents a difficult task. The functions of the various cellulase components are not completely understood, and different cellulase preparations may not be of the same component mixture. Also, the cellulase enzymes act synergistically and cellulase preparations may exhibit a difference in relative activities for different substrates. It is therefore important that the cellulase assay be based on a standard assay procedure with a model substrate that closely approximates the actual hydrolysis conditions.

A widely used test to measure the composite cellulase activity is the filter paper test. A 1 cm × 3 cm strip of Whatman #1 filter paper is the substrate. The filter paper is neither too susceptible nor too resistant to enzymatic attack and is universally available. This assay yields a measurement of the combined cellulase activities in terms of FPU. One FPU is the

amount of enzyme required to produce 1.0 μmol of reducing sugar per minute. (In some of the literature, however, the cellulase activity as defined here is termed an international unit [IU], and an FPU is defined as milligrams of sugar produced per hour.)

Three other substrates are widely used to measure the activity of subsets of the celluase complex. Cotton, a highly crystalline substrate, is used to determine the C_1 activity, or rather, the activity against crystalline cellulose. Carboxymethyl cellulose (CMC), a soluble derivative of cellulose, is used to measure the C_x activity, which is the activity against amorphous cellulose. Cellobiose, a soluble dimer of glucose, is used to measure the β-glucosidase activity.

Except perhaps for the β-glucosidase assay, none of the above-mentioned assays should be considered to determine the amount of a particular cellulase component. Due to the complex interactions present, these assays determine the combined activities of several enzymes.

SECTION 4.0

ENZYMATIC HYDROLYSIS PROCESS DESIGN

The enzymatic hydrolysis cellulose-to-ethanol process presented in this study is described in this section. The process flowsheet is the same as that described by Chem Systems Inc. (Chem Systems Inc. 1982a) with modifications to the enzyme production and enzyme hydrolysis sections. These modifications were made in order to implement recent results in enzyme production, enzyme hydrolysis, and enzyme recycle obtained from H. Blanch and co-workers at the Lawrence Berkeley Laboratories.

4.1 OVERALL PROCESS DESIGN

A major block diagram of the base-case process is presented in Figure 4-1. In this design, enzyme production, cellulose hydrolysis, and glucose fermentation are performed in separate vessels. Incoming biomass feedstock is sent through a steam explosion pretreatment. After steam explosion, a portion of the feedstock is sent to enzyme production and the remainder is sent to the hydrolysis section. Cellulase enzyme is produced via fed-batch fermentation in the enzyme production section and is sent to the hydrolysis section. After hydrolysis, the sugar-rich liquid stream is sent to fermentation. The ethanol-rich stream from fermentation is purified and dehydrated in the purification section. Residual cellulosics and lignins from the hydrolysis vessels are sent to a lignin boiler to provide process steam. Yeast and cell mass from the fermentation and enzyme production sections are sent to the by-product recovery section for sale as single-cell protein (SCP). Stillage from the distillation section is sent to waste disposal.

Figure 4-2 is a block diagram for a process that includes enzyme recycle. Recycle is accomplished by adsorption of the soluble cellulase enzyme in the hydrolysate onto fresh biomass feedstock. This process differs from the one depicted in Figure 4-1 only in that the hydrolyzate liquors are directed through a two-stage contacting reactor prior to being sent to concentration and fermentation. A portion of the fresh hydrolysis biomass feed is directed through this contacting reactor after steam explosion.

4.2 DETAILED PROCESS DESIGN

4.2.1 Pretreatment

The base-case process design incorporates steam explosion as a pretreatment. A block flow of this section is presented in Figure 4-3.

The biomass feedstock is assumed to be supplied in a form suitable for direct feeding into the steam explosion guns. In the case of a hardwood feedstock this assumption implies that the wood has been chipped prior to delivery and that the chipped wood requires no further size reduction, such as milling.

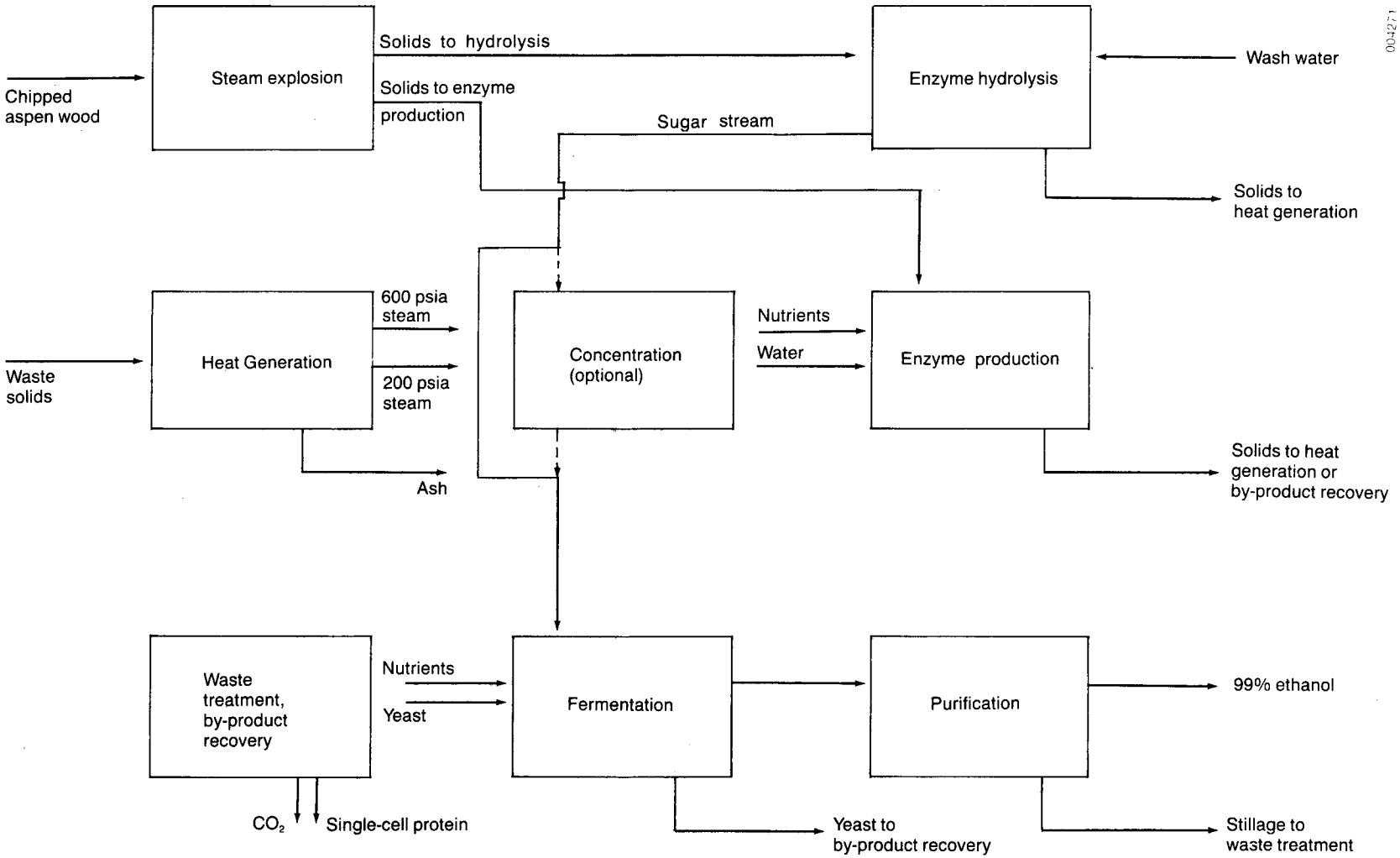


Figure 4-1. Enzyme Hydrolysis Process Block Diagram

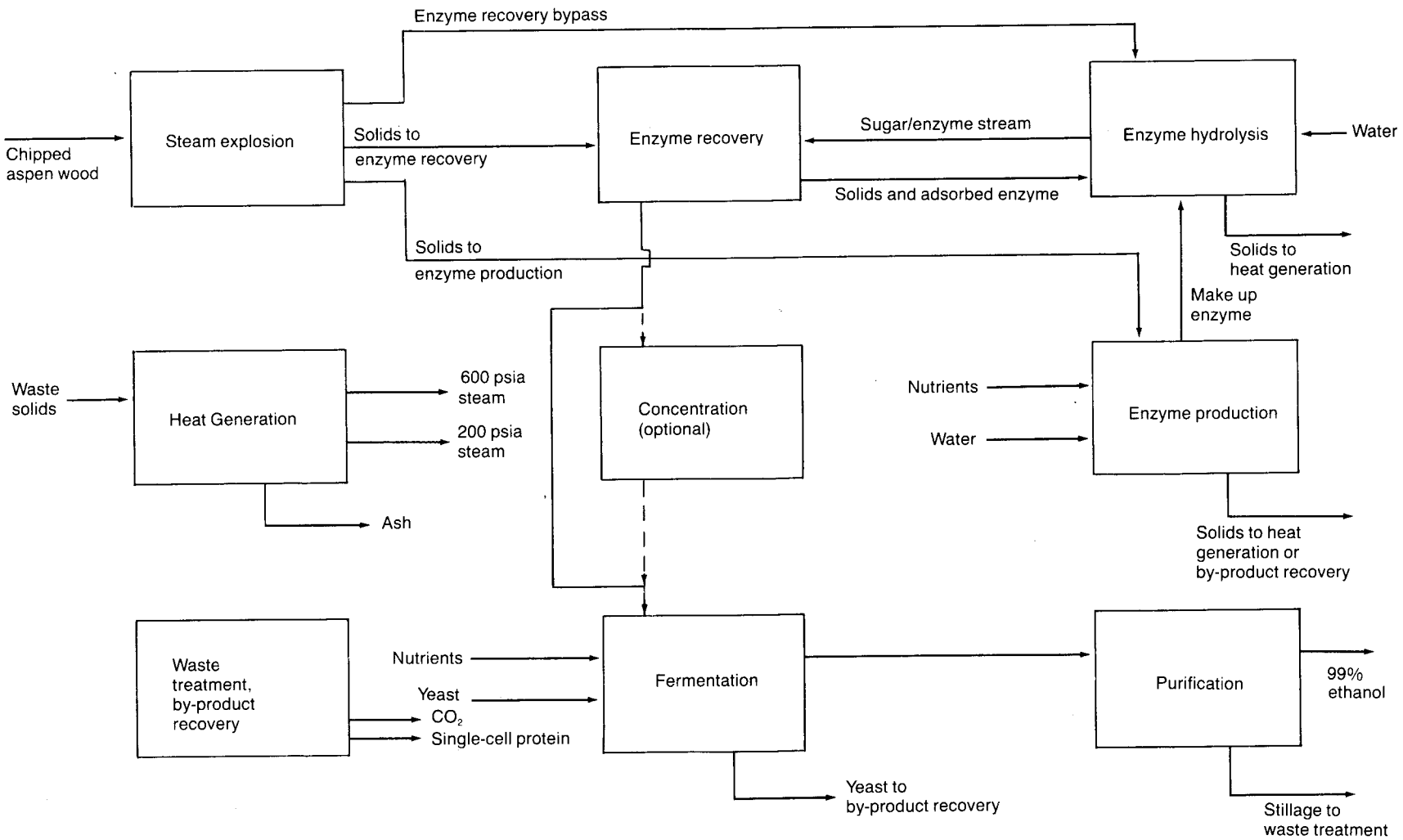


Figure 4-2. Process Block Diagram Including Enzyme Recovery

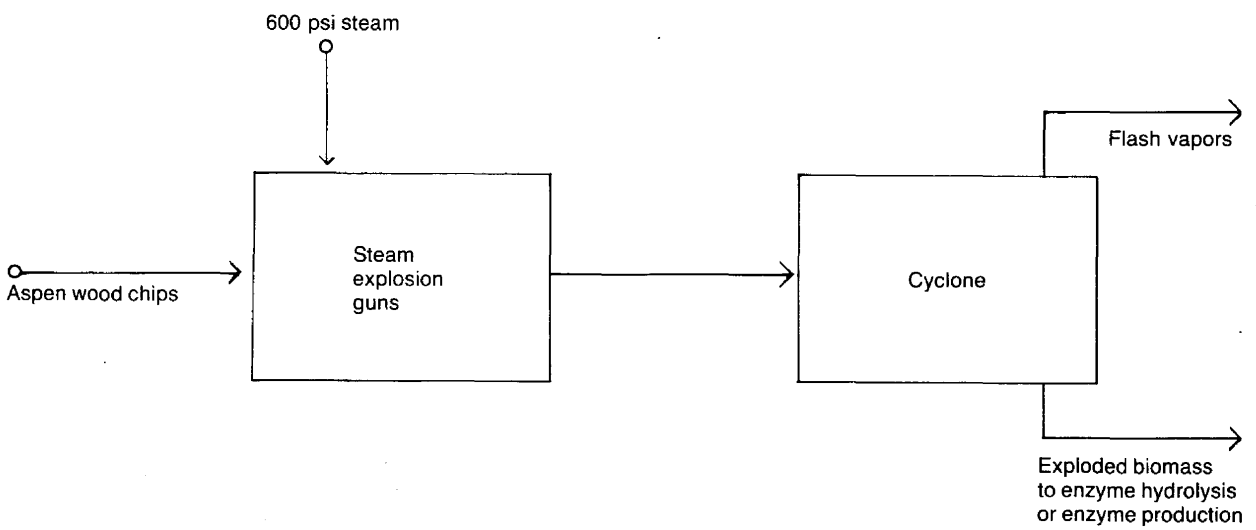


Figure 4-3. Steam Explosion Block Diagram

The steam explosion process developed by Iotech is a batch process. An explosion gun is charged with biomass and steam is injected until the desired cook temperature is attained. After the required cook time, the reactor pressure is quickly reduced, explosively discharging the contents into a cyclone. Some water and degradation products are flashed in the cyclone, and the resultant product is sent on to enzyme production or hydrolysis.

Chem Systems Inc. analyzed the Iotech experimental results and determined the kinetics of hemicellulose conversion for the following operating conditions:

Steam: 400 lb/ton of feedstock, 560 psig, 247°C
Cook time: 5 seconds.

Recent information obtained from researchers involved with the Iotech process indicates that the steam requirements per ton of wet feedstock will be much greater than the 400 lb/ton used in this report. Heat reuse to preheat the incoming feed will reduce these estimates, but 400 lb/ton may indeed be overly optimistic.

The equations for hemicellulose conversion and product formation are listed in Table 4-1 (Chem Systems Inc. 1982b). In determining these equations, Chem Systems made the following assumptions:

- Iotech experimental data (Iotech Corporation 1980) form the basis for the xylose and degradation product conversions from hemicellulose and cellulose.

Table 4-1. Steam Explosion Reaction Equations Developed by Chem Systems Inc.

Xyloses produced from pentosans:
0.0018 × hemicellulose (lb) × 0.711 = xyloses produced (lb)
0.88 × xyloses produced (lb) = pentosan consumed (lb)
0.12 × xyloses produced (lb) = H_2O consumed (lb)

Degradation products produced from pentosans:
0.013 × hemicellulose (lb) × 0.711 = pentosan consumed to degradation products (lb)
0.136 × pentosan consumed (lb) = H_2O consumed (lb)
1.136 × pentosan consumed (lb) = degradation products formed (lb)

Furfural produced from pentosans:
y × 0.64 × degradation products formed (lb) = furfural formed (lb)
0.36 × degradation products formed (lb) = H_2O formed (lb)

Pseudolignin produced from pentosans:
Z × 0.813 × furfural formed (lb) = pseudolignin formed (lb)
0.187 × furfural formed (lb) = H_2O formed (lb)

Degradation products produced from hexosans:
0.013 × hemicellulose (lb) × 0.289 = hexosan consumed to degradation products (lb)
0.11 × hexosan consumed (lb) = H_2O consumed (lb)
1.11 × hexosan consumed (lb) = degradation products produced (lb)

HMF produced from hexosans:
0.7 × degradation products produced (lb) = HMF produced (lb)
0.3 × degradation products produced (lb) = H_2O produced (lb)

Pseudolignin produced from hexosans:
0.86 × HMF formed (lb) = pseudolignin formed (lb)
0.14 × HMF formed (lb) = H_2O produced (lb)

- Hemicellulose and cellulose are converted to sugars and degradation products according to the following reactions:

$$\text{pentosan} \xrightarrow{+ H_2O} \text{xylose} \xrightarrow{- 3\ H_2O} \text{furfural} \xrightarrow[+ \text{lignin}]{- H_2O} \text{pseudolignin}$$

$$\text{hexosan} \xrightarrow{+ H_2O} \text{glucose} \xrightarrow{- 3\ H_2O} \text{HMF} \xrightarrow[\text{lignin}]{- H_2O} \text{pseudolignin}\ .$$

- No net glucose is formed within the operating range studied (5 to 32 seconds cook time) and the decomposition of cellulose does not occur prior to 60 seconds cooking time.

- The weight percent of hemicellulosic hexosans converted to degradation products is equal to pentosans converted to xylose and degradation products.

- The xylose formation from hardwoods is the same as from corn stover, and the equations listed in Table 4-1 apply to either substrate.

4.2.2 Enzyme Hydrolysis

Figure 4-4 presents a diagram of the enzyme hydrolysis section. The hydrolysis reactors are constructed of carbon steel and are rated for atmospheric use. To facilitate high biomass loading, the hydrolysis feed is split and fed in up to four feed stages. Due to the decrease in slurry viscosity during the initial hours of hydrolysis, this allows a greater loading of biomass than if one feed stage was used. The following staging sequence is used:

Substrate Loading (wt %)	Number of Feed Stages
less than 10%	1
10% to 15%	2
15% to 20%	3
20% to 25%	4

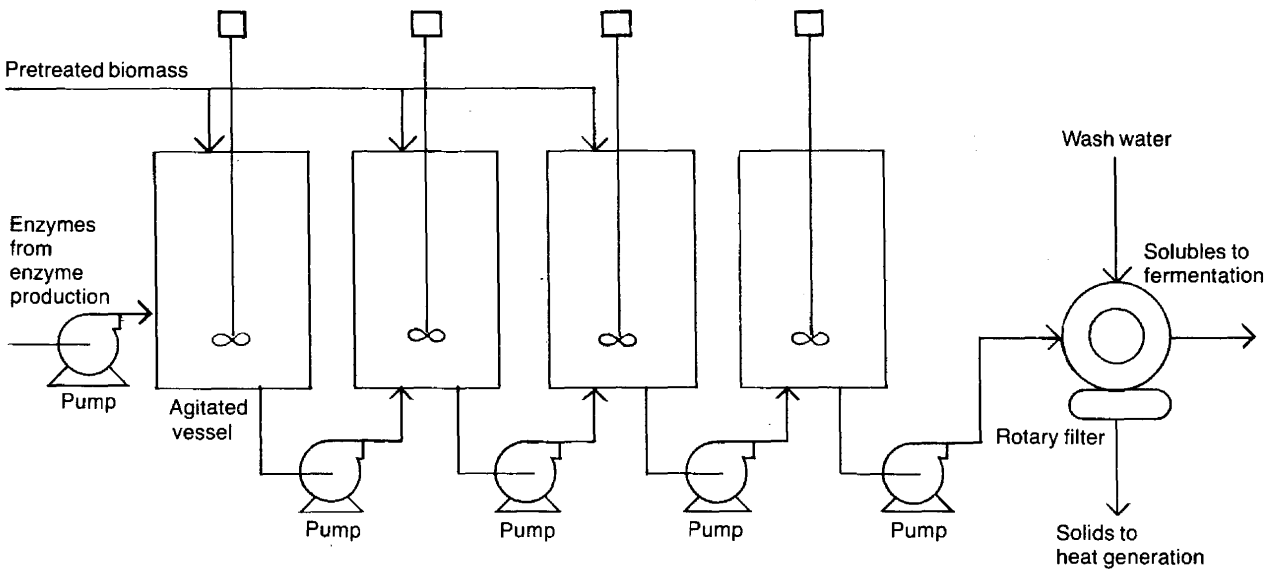


Figure 4-4. Hydrolysis Section (three feed stages)

For the base-case design, the hydrolysis section is run with a solids loading of 20 wt %, with 3 feed stages, at 45°C and pH 5.0, and with a total residence time of 48 hours.

Enzymes produced in the enzyme production section are continuously fed along with process water to the first hydrolysis feed stage. After hydrolysis, the slurry is washed in a rotary wash filter. Residual solids are sent to the lignin boiler to provide process steam. The glucose-rich hydrolysate is sent either to fermentation or concentration.

At present, a well-defined kinetic model of the hydrolysis reaction allowing a determination of cellulose conversion based on the parameters of feedstock, pretreatment, residence time, and enzyme loading is not available. From recent data it has been assumed that 80% cellulose conversion to glucose can be achieved at the base-case design parameters described above with an enzyme loading of 25 FPU/g of cellulose.

4.2.3. Enzyme Production

The enzyme production section is based on the design reported by Perez (1981). Modifications have been made to the operating conditions as reported by Perez to incorporate recent results on fed-batch enzyme production (Hendy 1981, Blanch 1983).

A schematic of the enzyme production section is shown in Figure 4-5. The enzyme is produced batchwise from the RUT C30 strain of *Trichoderma reesei* in fermenters constructed of 304 stainless steel, rated to 2.5 atm to enable steam sterilization. Batch cycle time is 13.5 days, which includes 13 days fermentation and 0.5 day for charging, discharging, and sterilizations.

Inoculum for each batch is produced in two stages of inoculation fermenters. The second stage has a working volume of 10% of the daily production and the first stage has a working volume of 10% of the second stage. The medium described in Table 4-2 is mixed and sterilized continuously prior to entering the fermenter.

Steam-exploded biomass feedstock is washed on a rotary wash filter to remove soluble lignins. The washed biomass is then added in increments of 50 g/L to the batch fermenters until a final loading of 150 g/L is attained.

After fermentation the enzyme broth is washed on a rotary wash filter. The filtrate is stored in a surge tank to facilitate continuous introduction to the hydrolysis section. The solids, consisting of approximately 18 g/L of mycelia, 7.5 g/L of cellulose, and the biomass noncellulosic material, are either sent to by-product recovery or burned in the lignin boiler to provide process steam.

Laboratory tests on the fed-batch enzyme production process have produced enzyme activity as high as 32 FPU/mL with the RUT C30 *T. reesei* strain, using Solka Flok as a carbon source. Recent results indicate that this titre may be achieved also by using the less expensive biomass feedstock as carbon source (Blanch 1983). The base-case process described in this report assumes that an enzyme strength of 30 FPU/mL can indeed be attained using the biomass as carbon source.

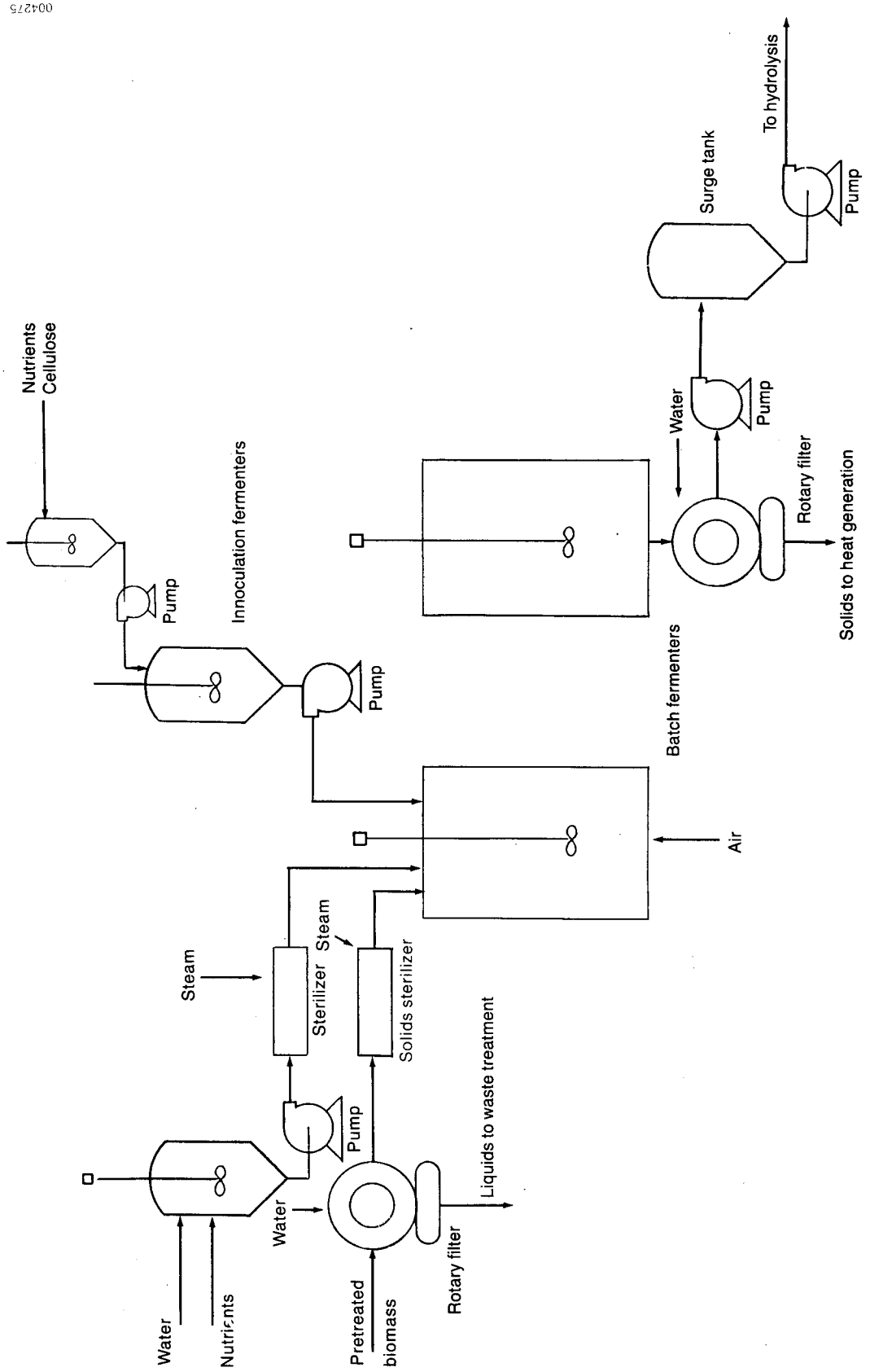


Figure 4-5. Enzyme Production Section

Table 4-2. Cellulase Production Medium

Component	Concentration (g/L)
$(NH_4)_2\ SO_4$	34.8
UH_2PO_4	11.4
$MgSO_4$	0.9
$CaCl_2 \cdot 2H_2O$	2.4
Corn Steep Liquor	8.7
Tween-80 (ml)	0.2
$FeSO_4 \cdot 7\ H_2O$	5.0
$MuSO_4 \cdot H_2O$	1.6
$ZnSO_4 \cdot 7\ H_2O$	1.4
$CoCl_2$	2.0

Medium is a variation on that presented in Perez 1981. The concentrations of the first 5 components above have been increased by a factor of 3 over that described by Perez for 50-g/L cellulose, due to the increase in cellulose concentration to 150 g/L (Blanch 1983).

4.2.4 Fermentation, Carbon Dioxide Recovery, Purification, and Offsites

The remaining process sections of fermentation, carbon dioxide recovery, and purification as well as the offsites are identical to the process reported by Wright (1982), and the following descriptions are exerpts from that report.

The fermentation block (Figure 4-6) consists of three parts: detoxification, fermentation, and carbon dioxide (CO_2) recovery. The ethanol stream produced in the continuous cascade fermenters is sent to purification. Carbon dioxide is recovered, liquefied, and sold as a by-product. A yeast purge stream is sold as single-cell protein animal feed.

The neutralized sugar solution is passed through columns of activated carbon to remove HMF, furfural, and any other trace hydrolysis degradation products that may be toxic to the yeast.

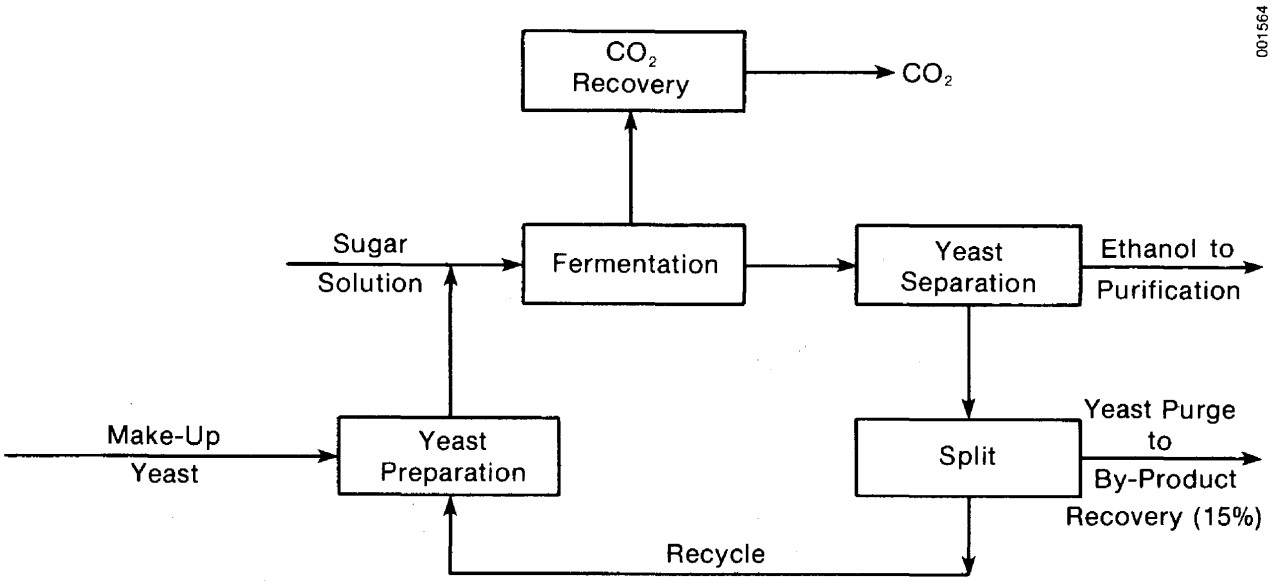


Figure 4-6. Fermentation Section

Fermentation is carried out at 30°C (85°F) in a continuous cascade scheme. The fermentation time is 24 hours. Ninety-five percent of the glucose is converted by the yeast to ethanol and carbon dioxide by the following reaction:

$$\underset{(180)}{C_6H_{12}O_6} \rightarrow \underset{(46)}{2C_2H_5OH} + \underset{(44)}{2CO_2}$$

In this reaction, 51 wt % of the glucose is converted to ethanol and 49% to CO_2. Three percent of the glucose is converted to glycerol, and 2% is converted to yeast. The yeast grows 10% during the cycle. Eighty-five percent of the yeast is recycled to the reactor, while the remainder is sold as a single-cell protein by-product. The concentration of yeast in the fermenter is augmented with fresh yeast to 9% of the total glucose input. The xylose passes through the fermenter unchanged.

The fermenters used are closed vessels. The CO_2 produced is collected, liquefied, and sold. The collected CO_2 is scrubbed with water to remove soluble impurities and then compressed to 2.02 MPa (300 psia). The compressed gas is passed through beds of activated carbon to remove any remaining impurities. The gas is then chilled, dried on a desiccant bed, and liquefied.

The ethanol-water stream from the fermenter is concentrated to the 94 wt % ethanol/6% water azeotrope in the beer still and then dehydrated to produce anhydrous ethanol by a ternary benzene distillation (Figure 4-7). The aqueous stillage (mainly water and xylose) is sent to the waste ponds. Steam usage is minimized by heat integration.

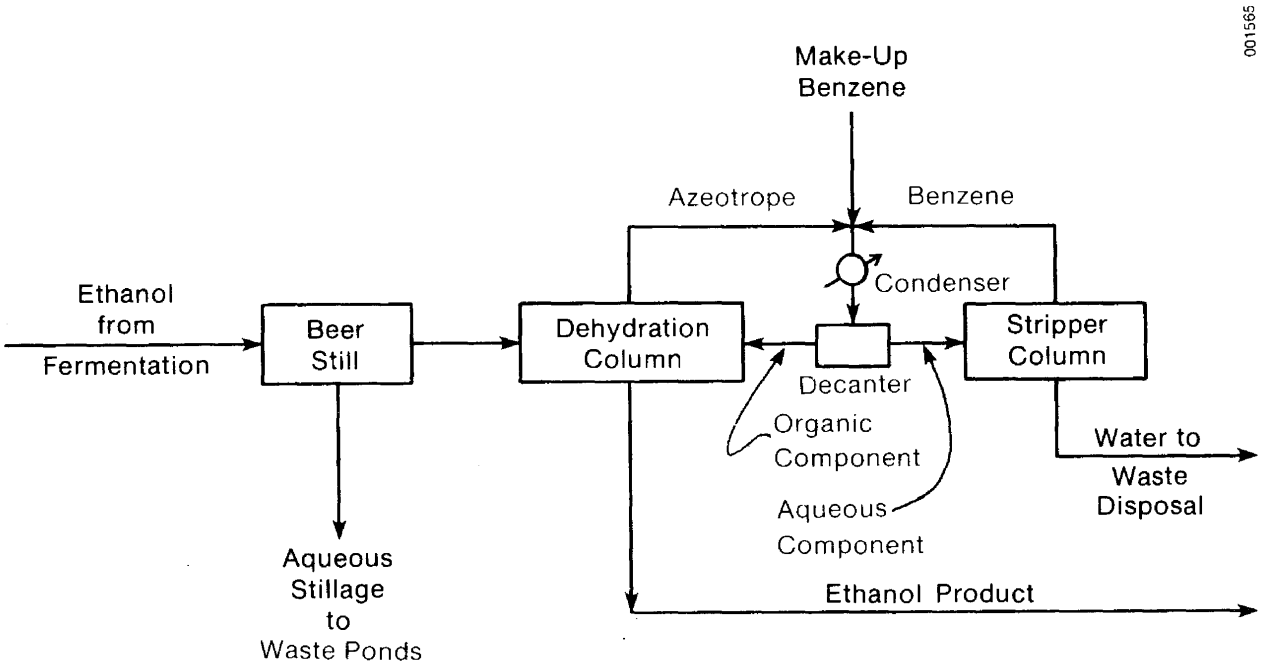


Figure 4-7. Purification Section

The beer feed from fermentation enters the rectification column (beer still). Heat to run the beer still is provided by 1.3 MPa (200 psia) steam. The upper part of the column utilizes sieve trays while the bottom uses disk and doughnut trays to handle the water, xylose, and suspended solids. The water stream (stillage) is sent to waste disposal. The column overhead stream contains the binary water-ethanol azeotrope.

The overheads from the beer still enter the dehydration column where benzene ternary distillation is used to break the azeotrope. Pure ethanol is removed from the bottom of the dehydration column. The dehydration column overhead stream is a tertiary azeotrope of ethanol, water, and benzene. The water is separated from the benzene and ethanol in a stripper column. The heat required for the dehydration and stripper columns is cascaded from the beer still, and the azeotropic distillation therefore does not require additional energy.

The offsites section includes heat generation, waste disposal, storage for raw materials and products, and the various utilities.

In the heat generation block the unreacted solids from the hydrolysis reactor (mainly lignin and unreacted crystalline cellulose) are neutralized, dried to 50% solids, and burned in the lignin boiler to produce 4.04 MPa (600 psia) steam. The lignin boiler is sized to dispose of the entire hydrolysis purge stream. The high pressure steam provides heat for the hydrolysis reactor. Excess steam is throttled to 1.35 MPa and used to heat the prehydrolysis reactor and distillation section, and to dry the boiler feed. If excess steam is available after the plant load is met, it is sold as a by-product at a price competitive with steam generated by a coal boiler, or at a lower price based

on the value of the unreacted solid feed. If the lignin boiler is not large enough to supply the plant demand, a coal boiler with flue gas desulfurization is added to make up the difference. The boilers constitute the single largest capital investment in the plant.

The aqueous stillage and various condensed flash vapors are collected and sent to a waste treatment pond. Electrical and cooling water systems are sized to meet the plant load. Storage for ethanol, calcium hydroxide, sulfuric acid, feedstock, and by-products is sized to accommodate two weeks of these materials.

4.2.5 Enzyme Recovery

Enzyme recovery via countercurrent adsorption has been included as a process option. A schematic of the equipment for this section is shown in Figure 4-8.

Enzyme recovery is accomplished by adsorption of the soluble cellulase enzymes contained in the hydrolysate stream onto pretreated biomass feed. Two contacting stages are used, each with a residence time of 0.5 hour. Each stage consists of an agitated carbon steel tank, conveyor, rotary filter, and pumps.

Laboratory results have indicated that up to 80% of the hydrolysis section filter paper activity may be released back into solution at high levels of cellulase conversion (Orinchowskyj 1982). Detailed computer modeling of the two-stage enzyme recovery section, using experimentally determined Langmuir isotherm constants for pretreated biomass to describe the enzyme adsorption, indicates that as much as 98% of this filter paper activity may be adsorbed onto the freshly pretreated biomass. Sixty percent enzyme reuse was therefore considered in the parametric analyses of Section 6.0.

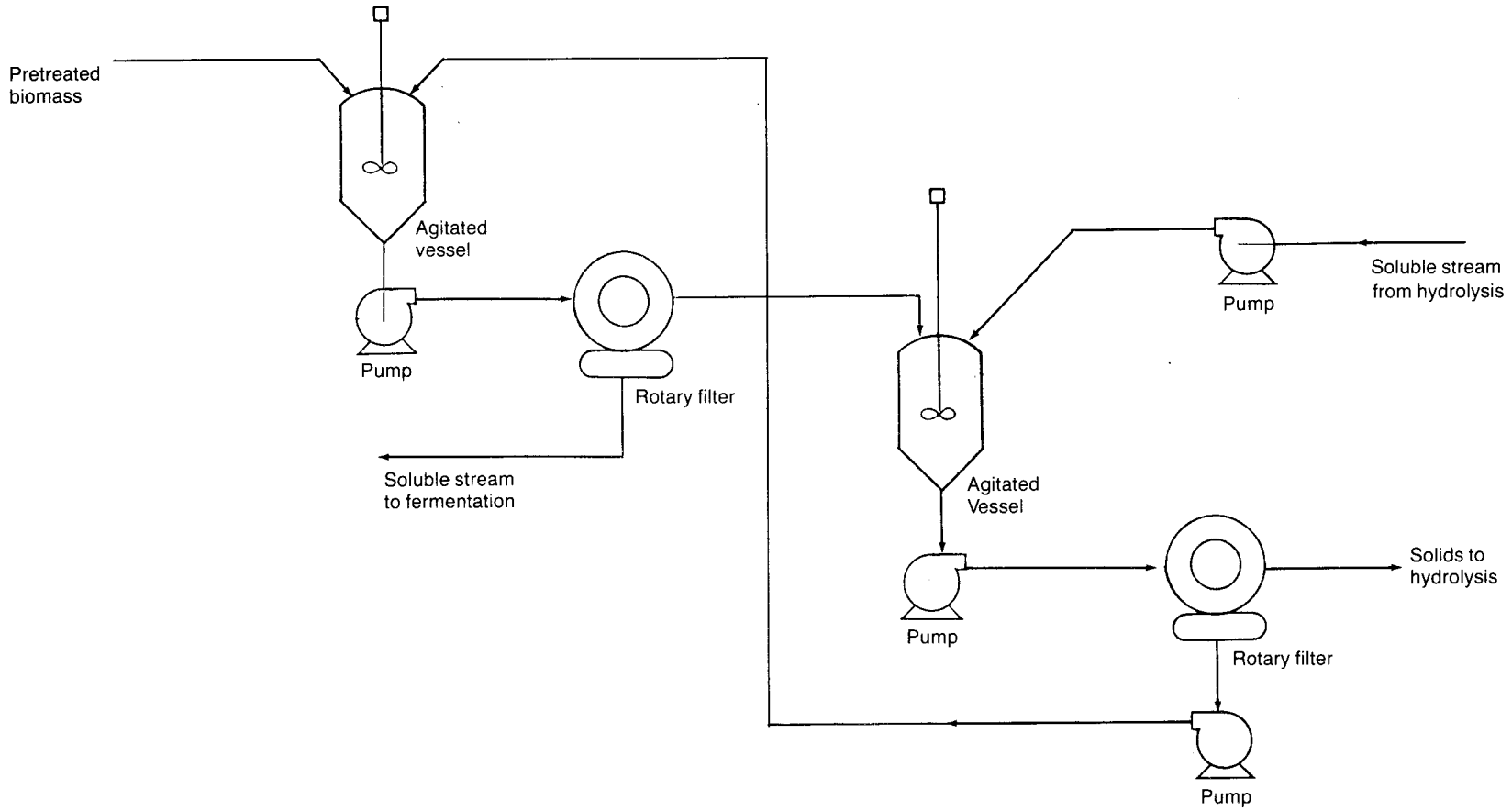


Figure 4-8. Two-Stage Countercurrent Enzyme Recovery Section

SECTION 5.0

SIMULATION MODEL

In order to determine the effect of operating conditions, process configuration, and assumed kinetics, it is desirable to model the operation of the entire biomass-to-ethanol plant. Earlier efforts have concentrated on the enzymatic hydrolysis portion of the process and modeled only on the basis of selling price of glucose (Perez 1981; Wald 1981). However, this method does not allow an integration of offsites (i.e., utilities and waste disposal) between the glucose production portion (front end) and the subsequent concentration, fermentation, and purification portion of the plant (back end).

The calculations presented in this report were executed by a simulation model developed by Chem Systems Inc., under subcontract to SERI. This model simulates the entire plant and determines a selling price for ethanol at the desired return on investment. Some modifications were made to the Chem Systems model as described in the previous section and will be discussed below, along with a brief description of the Chem Systems version. A more detailed documentation of the unmodified Chem Systems model can be found elsewhere (Chem Systems Inc. 1982).

The simulation model was developed for implementation on an IBM 5120 computer, using "A Programming Language" (APL). Minor modification allowed the model to be also implemented on a Control Data Corporation 720 mainframe computer, allowing for quicker execution time and direct data plotting.

Figure 5-1 shows the flow path of the simulated model. The first block, called EDITENZ, simply loads in operational data for the current run. ENZ1, the second block, calculates all stream flows and material balances. Once these flows have been established, they are scaled up in ENZ2 such that the desired production rate is achieved. ENZ3 calculates the capital cost of equipment based on major stream flows. The last block, ENZ4, determines the plant economics and the selling price of ethanol.

Capital cost for each major piece of equipment is determined based on the equation

$$\text{Cost} = \text{base cost} \times \left(\frac{\text{Size}}{\text{base size}}\right)^{\alpha} .$$

The base cost and base size were determined by Chem Systems Inc. for a 50 million gal/yr plant using the Icarus Cost Program developed by the Icarus Corp. of Rockville, MD. The exponent α was determined for each equipment type also using the Icarus Cost Program by perturbation of the base-case size and plotting of the resultant costs. Costs of equipment for the enzyme hydrolysis, enzyme production, and enzyme recovery sections were determined from costing estimates as described by Perez (1981).

The discounted cash flow (DCF) analysis used in ENZ4 to calculate the selling cost contains the following assumptions (from Chem Systems Inc. 1982):

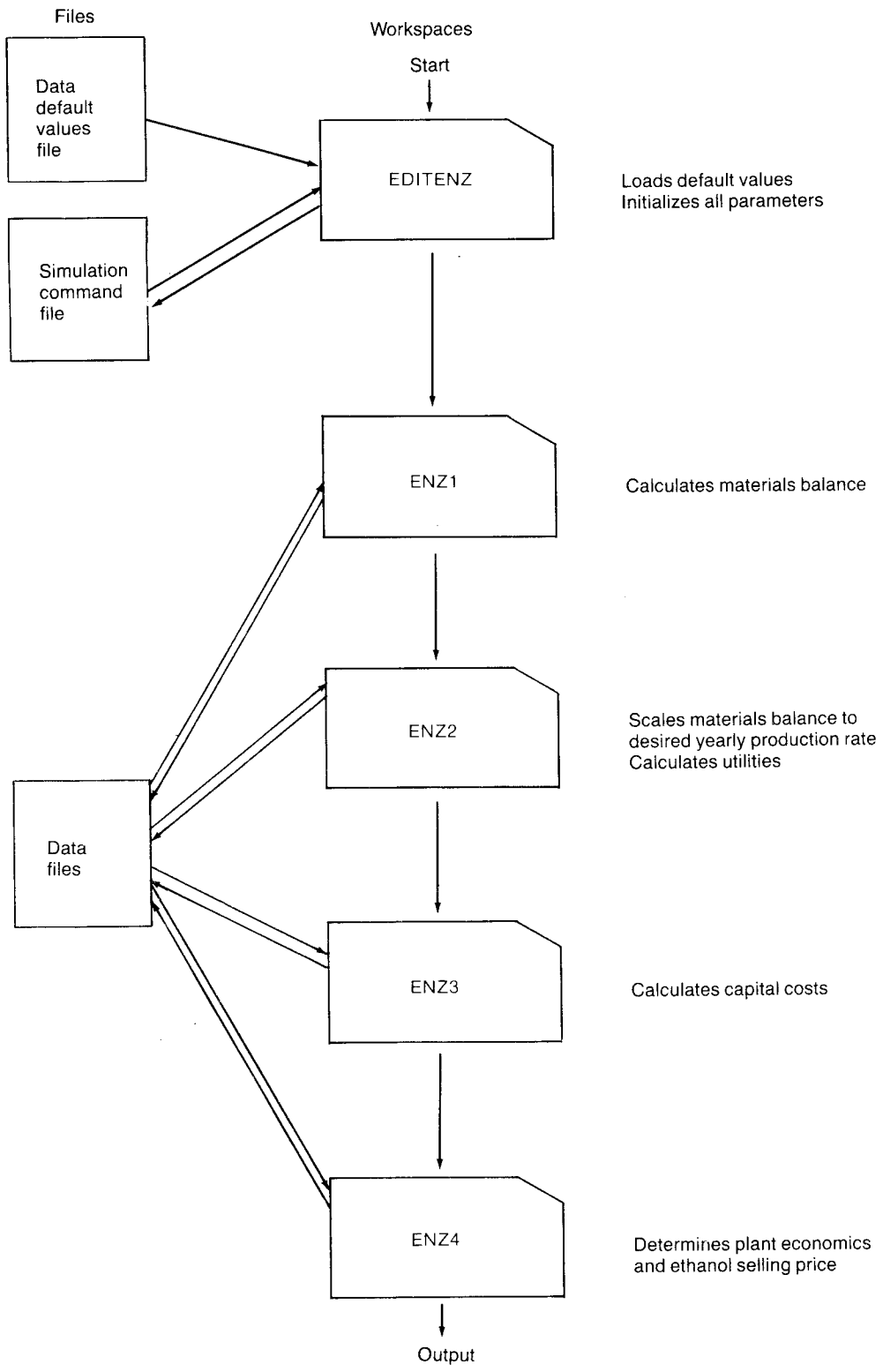


Figure 5-1. Simulation Model Block Diagram

- Plant basis is first quarter 1982 at a U.S. Gulf Coast location.
- Time of construction is two years with uniform expenditure of capital throughout this period.
- Working capital is the sum of
  Feedstock inventory--0.5 month of raw materials at delivered price
  Finished product inventory--0.5 month of products and by-products
  Accounts receivable--one month gross cost of production
  Cash--one month expenses (gross cost of production less depreciation)
  Warehouse/space parts inventory--3% of ISBL capital cost
  Less:
  Accounts payable--one month of raw materials at delivered prices.
- Total federal and local taxes is 50%.
- Depreciation is straight line, over a 5-year period for onsite investments and a 10-year period for offsite investments, with no salvage value for the plant.
- Cost of sales is 6% of the product selling price.
- A gradual sales buildup of 60% of capacity in the first year, 80% in the second, and 100% return from the third year on.
- Return on investment of 15%.

Modifications to the Chem Systems model were made to the subroutines performing the enzyme hydrolysis and enzyme production materials balances and costs. Additionally, subroutines were added to implement enzyme recycle via countercurrent adsorption. Material balance calculations for these sections are based upon the process descriptions presented in Section 4.0. Capital costs of equipment for these sections are determined as described by Perez (1981) and are based upon cost estimates found in the literature.

This simulation model is limited in its ability to determine an exact selling price of ethanol due to (a) lack of detail in the model and (b) lack of a full understanding of kinetics and processing constraints.

Regarding the first reason, the purpose of the Chem Systems model was to provide a method of comparison of process configurations and assumed kinetics, to aid in understanding major cost considerations, and to provide a direction for further research. The model was designed to provide not a detailed but rather a realistic estimate of the cost to produce ethanol. Particularly, major attention in the model is focused on the front end of the plant, the biomass hydrolysis, and lesser detail has been provided for the back end, ethanol production from the hydrolysis sugars.

Regarding the second reason, kinetics and materials handling processes are not well understood. Currently, there are no kinetics models to predict glucose conversion and ethanol fermentation that encompass all the possible variations in pretreatment conditions, feedstock, enzyme source, enzyme loading, hydrolysis conditions, and the presence of toxins. Also, the factors involving materials handling and exact costs and operating conditions for novel processes (e.g., steam explosion) are not completely known at this time.

The simulation model used for this report requires as input values for glucan conversion in the enzymatic hydrolysis section as well as for all operating conditions such as residence time and enzyme loading. It is up to the user to realize that the model does not contain a kinetic equation for enzymatic hydrolysis and that in order to obtain a realistic ethanol cost estimate, the user must input values based on realistic laboratory and pilot-plant results.

SECTION 6.0

PROCESS SIMULATION STUDIES

6.1 BASE-CASE DESIGN

Plant capacity was set at 50×10^6 gal of ethanol per year. Economies of scale usually dictate that the ethanol cost would decrease with increased plant capacity. However, with a biomass feedstock, increased plant capacity results in increased feedstock cost, due to increased collection radius. As discussed in an earlier report (Wright 1982), 50×10^6 gal/yr may be the largest capacity plant that can be reasonably built.

Aspen wood was chosen as the feedstock. A hardwood was chosen over an agricultural residue, such as corn stover, because of the inclusion of steam explosion as pretreatment. Currently available data on the steam explosion process do not indicate a change in operating conditions to effect a similar enzymatic conversion for different feedstocks. Due to the high cost of steam explosion, hardwoods may be more economical because they have a higher glucan content than agricultural residue (50% cellulose as compared with 35%-40% for corn stover on a dry basis). The cost for aspen wood was assumed to be $30/dry ton, or $0.75/wet pound, assuming 50% moisture content. The wood was assumed delivered to the plant site in a chipped form suitable for steam explosion without additional size reduction.

Since a comprehensive kinetic model for cellulose hydrolysis encompassing all the various parameters such as pretreatment, nature of substrate, and hydrolysis conditions is not available, an estimation must be made of the percentage of cellulose-to-glucose conversion for a given set of operating conditions. Recent laboratory results have indicated conversions in excess of 80% resulting in sugar solutions in excess of 12 wt % using steam-exploded biomass (Blanch 1983). For the base-case process, 80% cellulose-to-glucose conversion was assumed, with a residence time of 40 hours, solids loading of 20 wt %, and an enzyme loading of 25 FPU/g of cellulose.

This combination of 80% conversion and 20 wt % solids loading produced a glucose stream of approximately 10 wt %, which eliminated the need for a concentration step prior to fermentation. This was shown by simulation of the base-case process while incorporating the concentration step. The selling price of ethanol is $2.39/gal with concentration to 20 wt % glucose solution prior to fermentation, and $2.13/gal with no concentration. The savings in purification and fermentation capital and utilities when concentration is incorporated are less than the increased costs associated with the multieffect evaporator. However, a more detailed design of the waste treatment section than is currently available in the model may alter these results.

Recent laboratory results have indicated that as high as 32 FPU/mL enzyme titre can be produced from RUT C30 using corn-steep liquor as a nitrogen source and Solka Flok at a level of 150 g/L as a carbon source, in fed-batch fermentations (Blanch 1983). Batch residence time was approximately 13 days. Recent results also indicate that similar high enzyme titres can be achieved using water-washed, steam-exploded biomass as the carbon

source (Blanch 1983). The base-case design of the enzyme production section assumes an enzyme strength of 30 FPU/mL produced in a 13.5-day cycle time, utilizing the washed, steam-exploded aspen wood as the carbon source.

The base-case design does not include enzyme recovery and reuse, and sends the solubilized xylose to the waste treatment ponds.

The production cost summary for the base-case design is shown in Table 6-1. For the 15% return on investment (ROI), a selling price of $2.13/gal is indicated.

The largest contributor to the net cost of production is depreciation, at 66.2¢/gal. Table 6-2 shows the summary of capital cost due to offsites and onsites. Column 2 shows the percentage contributions to onsite purchased equipment cost for each of the major processing steps. The most capital-intensive processing steps are steam explosion and enzyme production, accounting for 35% and 43% of the onsite purchased capital, respectively. Due to depreciation, these processing steps contribute 18¢ and 22¢/gal, respectively, to the net cost of production. It must be noted that with the economic analysis of this simulation model, overhead, operating costs, and offsite equipment costs are based in part on the onsite capital equipment cost, and therefore these two processing steps contribute heavily to the production costs in these areas as well.

The second largest contribution to ethanol cost is due to raw materials. Aspen wood costs dominate the raw material costs, contributing 46¢/gal.

Utilities account for 21¢/gal. Table 6-3 presents a summary of the utilities for each processing step. Power requirements are highest for enzyme production, accounting for 7.4¢/gal. Hydrolysis and fermentation also consume large amounts of power, contributing 3.3¢/gal each. Steam costs are not a major factor in the base-case costs, due to the onsite production of most of the process steam in the lignin boilers. The boiler costs contribute 5¢/gal to the depreciation costs. When utilities are considered together, however, steam consumption becomes an important factor, because any excess Btu value from residential biomass can conceivably be converted onsite to electrical energy. Electricity alone contributes 15¢/gal to ethanol costs. Steam costs also will become more of a factor if residual lignins are converted into a salable by-product, and are not available for process steam production.

Operating costs account for 19.6¢/gal. Of this cost, 84% or 16.4¢/gal are due to maintenance, which is calculated as a percentage of onsite capital cost, which, as shown above, is due mainly to the steam explosion and enzyme production steps.

6.2 PARAMETRIC ANALYSIS

This section presents the findings of a parametric analysis of the base-case process. First, a qualitative discussion provides the rationale for the chosen process variations. Next the results of single-parameter variations, along with some coupled-parameter variations are presented to illustrate their effects on the selling price of ethanol. The process component costs of

Table 6-1. Cost of Production Estimate for Ethanol Process-Enzyme Hydrolysis

Basis	Capital Cost Summary ($M)	
U.S. Gulf Coast Location	Battery limits	137.2
First Quarter 1982	Offsites	56.5
50.0 × 10^6 gal/yr capacity	Total fixed inventory	193.6
149,335 metric tons/yr	Working capital	15.3
8000 h/yr str. time		

Production Cost Summary

Component (units)	Units per gal of Ethanol	Price (¢/unit)	Annual Cost ($M)	Cents per gal of Ethanol	$/ Metric Ton
Raw materials					
Aspen wood (lb)	61.7198	0.8	23,145	46.29	
Sulfuric acid (lb)	0.0000	4.3	0	0.00	
Nutrients	0.6191	6.5	2,012	4.02	
Catalyst and chemicals			5,681	11.36	
Total raw materials			30,838	61.68	206.50
Utilities					
Power (kWh)	3.11395	4.6	7,162	14.32	
Cooling water (10^6 gal)	0.25803	7.3	942	1.88	
Process water (10^6 gal)	0.01634	65.0	531	1.06	
Boiler feedwater (10^6 gal)	0.00195	113.0	110	0.22	
Steam, 200 psia (10^6 lb)	0.00130	480.0	313	0.63	
Steam, 55 psia (10^6 lb)	0.00520	470.0	1,221	2.44	
Total utilities			10,279	20.56	68.83
Operating costs					
Labor, 46 men at $26,000			1,196		
Foremen, 9 men at $29,600			266		
Supervision, 3 man at $35,600			107		
Maintenance, material, and labor, 6% of ISBL			8,230		
Total operating cost			9,799	19.60	65.62
Overhead expenses					
Direct overhead, 45% labor and supervision			706		
General plant overhead, 65% operating costs			6,369		
Insurance and property tax, at 1.5% total fixed inventory			2,905		
Total overhead expenses			9,980	19.96	66.83
By-product credit					
Carbon dioxide (lb)	6.55120	2.8	-9,172		
Single-cell protein (lb)	0.40484	15.0	-3,036		
Total by-product credit			-12,208	-24.42	-81.75
Cash cost of production			48,688	97.38	326.03
Depreciation, 20% ISBL + 10% OSBL			33,080		
Net cost of production			81,768	163.54	547.54
Sales price at 15% DCF				212.6	711.8

Table 6-2. Base-Case Capital Cost Summary

	Cost ($)	% of Purchased Equipment
Purchased Equipment Costs		
Raw material handling	16,700,602	35.42
Enzyme production	20,093,196	42.61
Enzyme hydrolysis	3,869,203	8.21
Fermentation	2,648,985	5.62
Purification	2,323,179	4.93
Heat generation	1,515,542	3.21
Total Purchased Equipment Cost	47,150,707	100.00
Total Installed Equipment Cost	97,149,624	
Engineering and construction overhead	16,879,953	
Engineering fee and contingency	15,371,130	
CO_2 recovery system package	7,758,127	
Total plant ISBL cost	137,158,834	
Offsites Capital Cost		
Ethanol storage, 14 days	826,946	
Sulfuric acid storage, 14 days	968	
By-product storage, 14 days	241,804	
Yeast storage, 14 days	65,765	
Nutrient storage, 14 days	723,278	
Lignin steam boiler, 600 psia	19,690,764	
Steam boiler, 600 psia	5,510,085	
Cooling water system	2,416,167	
Electrical	5,060,175	
Buildings, 3% ISBL	4,114,760	
General utilities, 5% ISBL	6,857,942	
Site development, 3% ISBL	4,114,765	
Piping, 3% ISBL	4,114,765	
Pollution control, 2% ISBL	2,743,177	
Total offsites cost	56,481,367	
Total plant capital cost	193,640,201	

select process variations are then summarized, both with respect to their individual contributions and to various combinations. Finally, the sensitivity of the selling price of ethanol to feedstock cost is presented for a few alternatives to the base-case design.

Table 6-3. Base-Case Utilities Summary

Processing Step	Steam (1000 lb/h)				Power (hp)	Cooling Water (gal/min)	Process Water (gal/min)
	15 psia	55 psia	200 psia	600 psia			
Steam explosion	-73.4	0.0	0.0	101.9	385.7	0.0	0.0
Enzyme production	15.0	0.0	0.0	0.0	13,529.3	1,137.6	933.1
Enzyme hydrolysis	26.7	0.0	0.0	0.0	7,200.8	0.0	769.3
Fermentation	0.0	0.0	0.0	0.0	6,023.3	5,863.0	0.0
Purification	0.0	0.0	192.1	0.0	124.7	6,997.5	0.0
Heat generation	0.0	64.2	-184.0	-101.9	94.4	7,191.7	0.0
Waste treatment	0.0	0.0	0.0	0.0	2.0	5,688.4	0.0
	31.7	64.2	8.1	0.0	27,360.2	26,872.2	1,702.3

6.2.1 Qualitative Analysis of Process Improvements

One of the major contributors to the ethanol cost was shown to be feedstock costs. For a feedstock cost greater than zero, cost reduction can be achieved with an increase in feedstock utilization--either an increase in cellulose conversion or the utilization of xylose to produce additional ethanol. Approximately 7% of the pretreated feedstock is diverted to the enzyme production section, which accounts for 3.2¢ of the 46.3¢/gal feedstock cost. Substitution of this portion with a less expensive carbon source can perhaps be accomplished using whey, or in the case of development of a constitutive enzyme producer, with a soluble carbon source. This would increase feedstock utilization to produce ethanol as well as reduce the capacity of the steam explosion section.

Cost of production due to the steam explosion pretreatment was shown to be a major factor in the selling price of ethanol. Enhanced biomass utilization would decrease pretreatment capacity and thus lower costs. Substitution of the steam explosion process with a less costly pretreatment or a reduction in the estimated capital cost and steam requirements would reduce costs due to the pretreatment, but this reduction may be offset by any increases necessitated by lesser enzymatic conversions caused by less effective pretreatment.

A reduction in enzyme production costs can be achieved by decreasing the enzyme production capacity. This would be accomplished if it were possible to reduce enzyme loading per gram of cellulose, incorporate enzyme recovery, increase enzyme titre per batch, or reduce the batch cycle time. Laboratory results discussed in Appendix B indicate that up to 65% of the filter paper activity of the cellulase enzymes may be recycled and reused by adsorption onto the pretreated hydrolysis solids feed. Hydrolysis data shown in Appendix B indicate that enzyme loading may be decreased below 25 FPU/g perhaps at the expense of an increase in hydrolysis residence time. The base-case values for enzyme titre and batch time are at the best reported levels, and improvements here may be more speculative with the RUT C30 organism.

Capital cost for the enzyme production section is very high due to stainless steel construction and use of fermentors designed to withstand 2.5 atm and equipped with cooling coils. The base-case enzyme production section is designed as described by Perez (1981), and may be conservatively expensive for this section. Few data are available on large-scale studies of cellulase production, and this design is included as perhaps a worst case. Some pilot-plant data suggest that the enzyme production can be carried out in less expensive equipment (University of Arkansas 1981). A reduction of equipment cost for enzyme production would have a large effect on the ethanol price.

Additional salable by-products from lignins or xylose will also effectively increase the feedstock utilization and decrease the ethanol selling price. However, in the case of lignins, the by-product credit would be offset by the loss of fuel value to produce process steam. Fermentation of xylose to ethanol would allow a decrease in plant size for a given yearly ethanol production rate.

A decrease in hydrolysis residence time would decrease the costs due to hydrolysis capital and operating costs. An increase in hydrolysis solids loading

would decrease fermentation, distillation, and waste treatment costs due to the resulting higher concentration of glucose in the processing streams. However, it is expected that correlations exist between hydrolysis residence time, solids loading, and conversion, and that parametric analyses of one of these parameters with the others held constant may not represent a possible set of conditions.

6.2.2 Variations in Single and Coupled Parameters

Figure 6-1 shows the selling price of ethanol for levels of enzymatic recycle from 0% to 75%. At 75% enzyme recycle, the selling price was reduced 27% to $1.55/gal. Addition of the enzyme recycle section adds approximately $0.5 million in capital cost and increased power consumption. However, at 75% recycle, the capital cost of the enzyme production section decreases from $20 million to $5.4 million. Figure 6-1 also indicates that over this range, the enzyme production depreciation contribution decreases from 23.4¢/gal to 6.3¢/gal and the power requirements drop from 7.4¢ to 2¢/gal. Even at the more realistic value of 60% enzyme reuse, the data indicate a reduction in selling price of 21.2% to $1.67/gal.

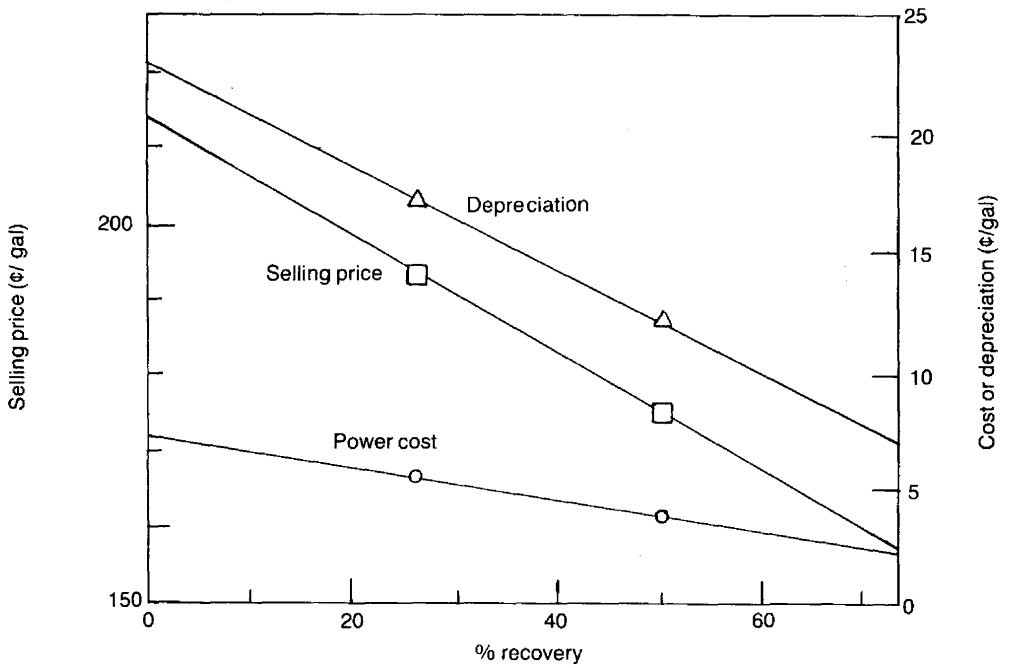


Figure 6-1. Variation of Selling Price, Depreciation, and Costs Due to Electricity with Percentage Enzyme Recovery and Reuse

To assess the effects of less costly enzyme production equipment, simulations were performed in which the equipment was taken as 25% to 100% of the base-case costs. Capital costs for enzyme production may be reduced if the fermentation can be performed in less complex equipment. Chemical rather than steam sterilization would negate the need for 2.5-atm rated vessels. External heat jackets would be less costly than internal cooling coils. Carbon steel construction, if feasible, would also reduce costs. Figure 6-2 shows the resulting selling price for a range of levels of enzyme recovery. For the case of no enzyme recovery, the selling price is reduced 19% to $1.73/gal at 25% of the base-case enzyme production cost. For this case, the enzyme production capital cost has been reduced from 43% to 15.7% of the total onsites capital.

Figure 6-2 shows that the effect of the reduction of enzyme production capital cost is reduced as enzyme production capacity is reduced, in this case by the use of enzyme recycle. At 75% enzyme recycle, the selling price is reduced to $1.45/gal at 25% enzyme production capital costs, but this is only a 7% reduction over the case of 75% enzyme reuse and base-case capital costs.

An increase in enzyme titre or a decrease in enzyme production batch time will similarly reduce enzyme production costs. Figure 6-3 shows the selling price of ethanol as a function of enzyme batch time. A 9.5-day cycle time reduces the price to $1.95/gal for a decrease of 8.1%, and a 5.5-day cycle time reduces the price to $1.78/gal for a decrease of 16.5¢/gal. Capital cost for both these cases is reduced from $20 million to $14.7 million and $9.1 million, respectively. Like Figure 6-2, Figure 6-3 shows that as enzyme reuse is incorporated, the relative effect of a decrease in batch cycle time is reduced.

Simulation runs were performed in which the enzyme loading was decreased from 25 FPU/g to 5 FPU/g of cellulose for various levels of enzyme recycle; the results are presented in Figure 6-4. In all these runs, the percentage of glucan conversion was held at the base-case value of 80%. It is unlikely that this level of cellulose conversion could be achieved at lower enzyme loadings without other process modifications such as a more efficient pretreatment or a large increase in hydrolysis residence time. These simulations merely illustrate the effect on costs produced by a reduction in enzyme loading alone.

When the enzyme load is reduced to 5 FPU/g, the selling price is reduced by 29.4% to $1.50/gal at 0% enzyme reuse and by 35.8% to $1.36/gal at 75% enzyme reuse. The major contributor to this reduction in price is the reduced enzyme production capital and operating costs due to reduced enzyme production capacity. A minor contribution is due to the reduction in feedstock cost and pretreatment costs. At 25 FPU/g and no enzyme recycle, approximately 7% of the feed is directed to enzyme production; but at 5 FPU/g and no enzyme recycle, this is reduced to 1.5%.

The base-case design assumes 80% conversion of cellulose at 40-hours residence time and base-case hydrolysis conditions. Variations in process parameters, such as pretreatment, feedstock, substrate loading, and enzyme loading, may alter the residence time required to make a particular conversion. The cost effect of the hydrolysis residence time was determined at various cellulose conversions.

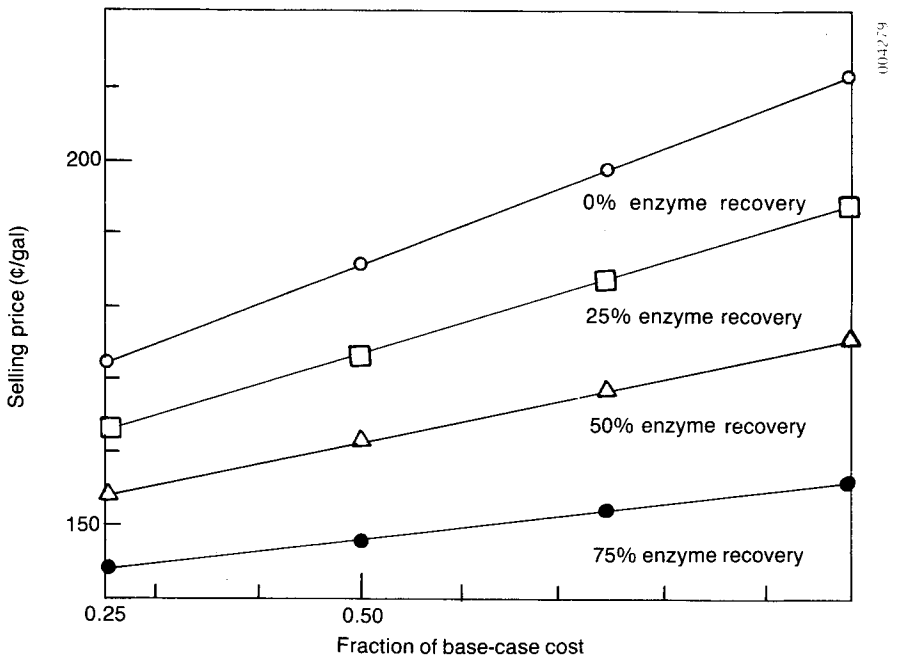


Figure 6-2. Ethanol Selling Price versus Capital Cost of Enzyme Production Equipment for Various Percentages of Enzyme Recovery

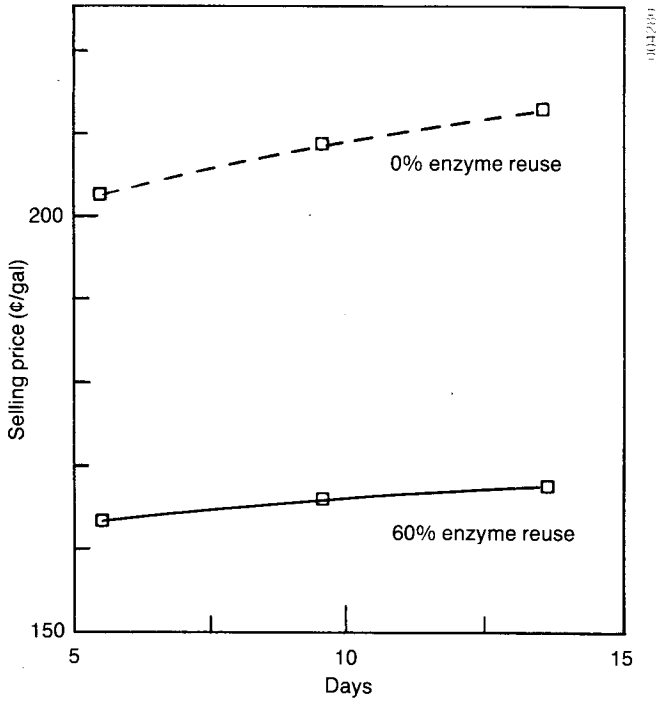


Figure 6-3. Ethanol Selling Price versus Enzyme Production Batch Time at Two Levels of Enzyme Recovery

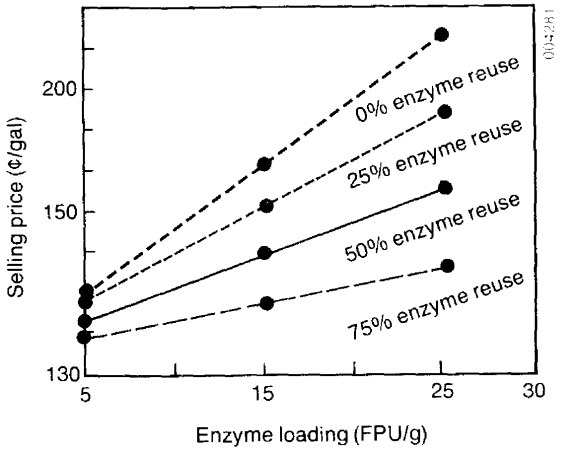


Figure 6-4. Ethanol Selling Price versus Enzyme Loading (per gram of cellulose) at Various Levels of Enzyme Recovery

At constant conversion, the selling price is not greatly affected by small reductions in residence time. This is to be expected, because changes in residence time alone affect only hydrolysis capital and operating costs, which are not major contributors to the cost of ethanol. Figure 6-5 shows that at 80% conversion, there is a reduction of only 4.7% to $2.03/gal at 12 hours. As the residence time for 80% conversion is increased by roughly a factor of 3 to 125 hours, the increase in price is 14% to $2.43/gal. This is due to an increase in hydrolysis capital cost to 20.7% of the onsites, for a total of $11.2 million purchased price. It must be noted that the effect of process improvements that would be intended to reduce production costs (e.g., enhanced cellulose conversion, lowered enzyme loading, less expensive pretreatments, etc.) may be somewhat offset if kinetics necessitated a significant increase in hydrolysis residence time.

Figure 6-6 illustrates the effect on selling price of the solids loading in the hydrolysis section at various cellulose conversions. At constant conversion, an increase in substrate loading results in a decrease in selling price. This is due to the smaller capacity of the hydrolysis, fermentation, and purification sections, which results from the processing of streams of greater concentration. An increase in the base-case loading from 20 to 25 wt % decreases the selling price by only 3.5% to $2.05/gal. At 2 wt % solids loading and 80% cellulose conversions, the resulting sugar product stream concentration is high. Lower solids loading and conversions would have a more pronounced effect on the selling price of ethanol due to fermentation and distillation costs of the more dilute streams.

Some interactions between conversion and solids loading are illustrated in Figure 6-6. For example, in order to achieve the base-case cost of $2.13/ gal, the solids loading could be as low as 13 wt % if at the same time (perhaps due to removal of product inhibitions) the conversion is increased to 90%.

Figure 6-7 shows results of simulations in which the cellulose conversion was varied from 60% to 100% at a variety of enzyme loads. For 25 FPU/g, the selling price varies from $2.58/gal at 60% conversion, to $1.84/gal at 100% conversion. Total plant capital costs decrease from $240 million to $165.7 million over this range of conversions, because of the change in capacity

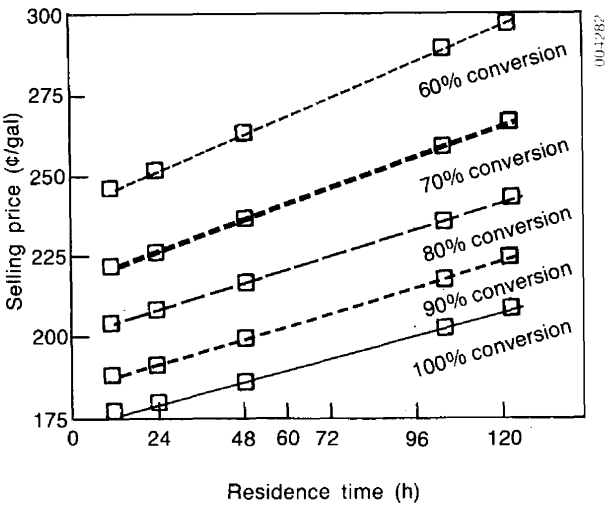


Figure 6-5. Ethanol Selling Price versus Hydrolysis Residence Time at Various Levels of Cellulose Conversion

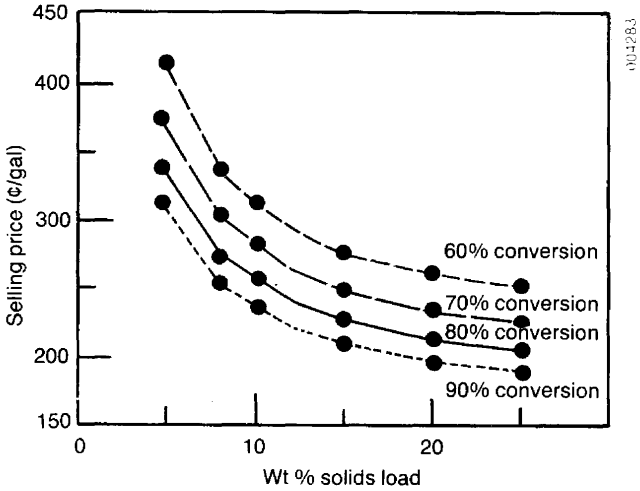


Figure 6-6. Selling Price of Ethanol versus Hydrolysis Solids Load at Various Levels of Cellulose Conversion

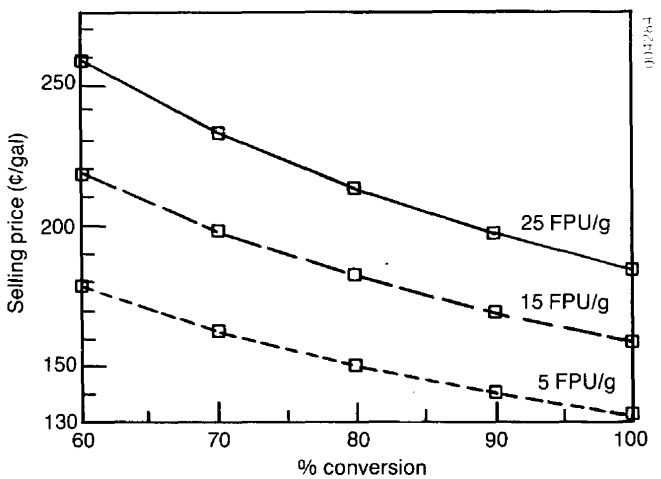


Figure 6-7. Ethanol Selling Price versus Cellulose Conversion at Various Levels of Enzyme Loading (FPU/g cellulose)

required to produce 50 × 10^6 gal/yr of ethanol. Utilities costs increase from 16.5¢ to 23.0¢/gal, due to less residual biomass available for plant steam production. Aspen costs decrease from 59.5¢ to 37.8¢/gal. An increase in the base-case conversion from 80% to 90% produces only a 15.8¢/gal decrease in selling price. The aspen costs decrease 10.2% from 46.3¢ to 41.6¢/gal.

Figure 6-8 shows the results of simulations in which the steam explosion capital cost and steam requirements were varied between 0% and 100% of the base-case values. As the costs associated with pretreatment decrease, so does the selling price. At 80% conversion, the selling price reaches a minimum of $1.74/gal at zero steam explosion

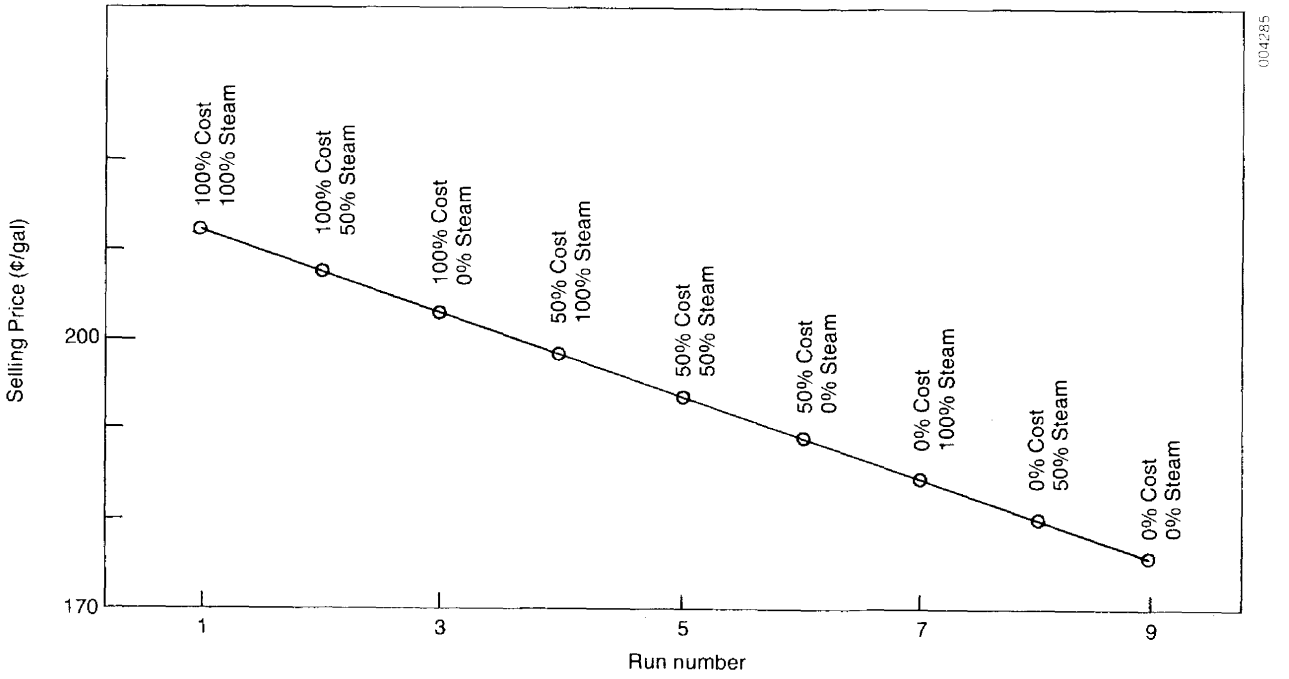


Figure 6-8. Ethanol Selling Price versus Various Levels of Base-Case Steam, Explosion Steam, and Capital Costs

costs. At only 50% reduction in both capital and steam usage, the price is reduced by 9.2% to $1.93/gal.

The base-case cost of $2.13/gal can be achieved where the costs due to pretreatment are practically zero (case 8, 0% capital and 50% steam usage) if 60% conversion can be attained. Reported conversions for milled, unpretreated biomass are below 60%, which illustrates the need for some form of pretreatment to enhance enzymatic susceptibility.

Substitution of steam explosion by a less costly pretreatment, or a redefinition of the steam explosion equipment and steam requirements, may decrease the ethanol selling price. However, as seen from Figure 6-8, enzymatic susceptibility is also a major factor to be considered in the pretreatment design.

When 90% of the available soluble xylose is "magically" converted to 5/6 its weight of glucose prior to fermentation, its selling price is reduced 17.2% to $1.76/gal. This simulation approximates a system in which an organism is available that can ferment both xylose and glucose with kinetics similar to that of glucose. Utilization of xylose enhances the feedstock utilization and decreases overall plant size. This results in the following reductions over the base case:

- From 61.7 to 48.7 lb aspen wood/gal ethanol
- From 46.3¢ to 36.5¢/gal aspen cost
- From 66.2¢ to 55.6¢/gal depreciation
- From $193.6 million to $164 million total plant capital cost.

6.2.3 Summary of Individual Parameter Variations

To evaluate the relative merits of individual process improvements, results of select simulations are tabulated, representing 11 process designs. The base-case design is included as the first design for comparison. The next seven designs represent the following individual changes from the base case:

- Inclusion of 60% enzyme reuse
- Fermentation of xylose
- Enzyme load decrease from 25 to 15 FPU/g
- Cellulose conversion increase from 80% to 90%
- Increase in hydrolysis solids loading from 20 to 25 wt %
- Reduction in pretreatment capital and utilities by 50%
- Reduction of enzyme production capital cost by 50%.

The final three designs represent combinations of these cases; the ninth design includes the first five improvements listed above, the tenth also includes 50% reduction in pretreatment costs, and the eleventh includes all seven of the improvements.

Selection of these seven improvements was based on the potential feasibility of implementation. Recent studies have indicated greater than 60% enzyme reuse may be possible with countercurrent adsorption (Orinchowskyj 1982). Currently much research is being undertaken to provide organisms that can ferment glucose and xylose. Many hydrolysis experiments have been performed with enzyme loadings at less than 25 FPU/g of cellulose (see Appendix B). Recent results have indicated that conversions of greater than 90% may be achieved with steam-exploded biomass (Blanch 1983). The cost of steam explosion is speculative and further pretreatment process research may reduce costs. Some pilot-plant data have indicated that enzyme production may be carried out in simpler, less expensive equipment. Finally, at high cellulose conversions, hydrolysis solids loading greater than 20 wt % may be possible with a staged method of feed introduction.

Reductions in hydrolysis residence time were not considered here. The residence time will be dependent on other, more cost-controlling parameters, such as enzyme load, pretreatment, desired conversion, and feedstock. As the change in selling price due to change in residence time is significant only at high residence times (as shown in Figure 6-5), residence time was considered of secondary importance to these other parameters.

Table 6-4 summarizes the cost components for each of the eleven cases. Columns 1 through 5 add up to the values in column 6, the cash cost of production. Column 8, the net cost of production, is the sum of depreciation, in column 7, and column 6. The selling price at 15% ROI in column 9 is calculated from column 8 by an algorithm in the Chem Systems simulation model. Column 10 shows the percentage decrease in selling price from the base-case price. Increasing the hydrolysis solids loading to 20 wt % only reduces the price 3.5% to $2.05/gal. The greatest single contribution to price reduction is seen to be addition of 60% enzyme recycle, which contributes a 21.2% reduction. Xylose utilization contributes 17.2% reduction.

Table 6-4. Cost Components (¢/gal)

		Raw Materials	Utilities	Operating Cost	Overhead	By-Product Credit	Cash Cost of Production	Depreciation	Net Cost of Production	Selling Price at15% ROI	Reduction in Selling from Base-Case Cost (%)
1.	Base case	61.7	20.6	19.6	20.0	-24.5	97.4	66.2	163.5	212.6	--
2.	60% enzyme recycle	54.4	15.5	15.3	15.9	-22.5	78.6	50.2	128.8	167.4	21.2
3.	Xylose utilization	50.1	19.4	16.8	17.3	-23.9	79.7	55.6	135.4	176.0	17.2
4.	15 FPU/g enzyme loading	56.8	17.1	16.7	17.2	-23.2	84.6	55.3	139.8	181.8	14.5
5.	90% cellulose conversion	56.1	21.9	18.2	18.6	-24.2	90.6	60.8	151.4	196.8	7.4
6.	25 wt % solids load	62.1	16.9	19.3	19.6	-24.5	93.4	64.3	157.8	205.1	3.5
7.	50% reduction of steam explosion cost	61.7	17.7	17.6	17.9	-24.4	90.5	58.1	148.6	193.1	9.2
8.	50% reduction of enzyme production cost	61.7	20.6	15.9	16.5	-24.4	90.2	52.8	143.0	185.9	12.6
9.	Combined cases 2-6	40.1	13.2	11.5	12.1	-21.9	55.0	35.5	90.5	117.6	44.7
10.	Combined cases 2-7	40.1	11.2	10.2	10.8	-22.0	50.3	30.0	80.2	104.3	50.9
11.	Combined cases 2-8	40.1	11.2	9.4	10.1	-22.0	48.8	27.3	76.1	99.0	53.4

The three combined-parameter cases show a decrease in selling price of 44.7%, 50.9%, and 53.4%, for selling prices of $1.18, $1.04 and $0.99/gal, respectively. All three cases represent significant cost reductions over the base case. The selling prices of column 9 are shown in bar chart form in Figure 6-9.

Table 6-5 provides a breakdown of the cents-per-gallon contribution to depreciation of the capital cost components for these 11 cases. Recycle of enzymes significantly reduces enzyme production capital (from 22.1¢ to 8.9¢/gal), slightly reduces the steam explosion capital, and produces the greatest

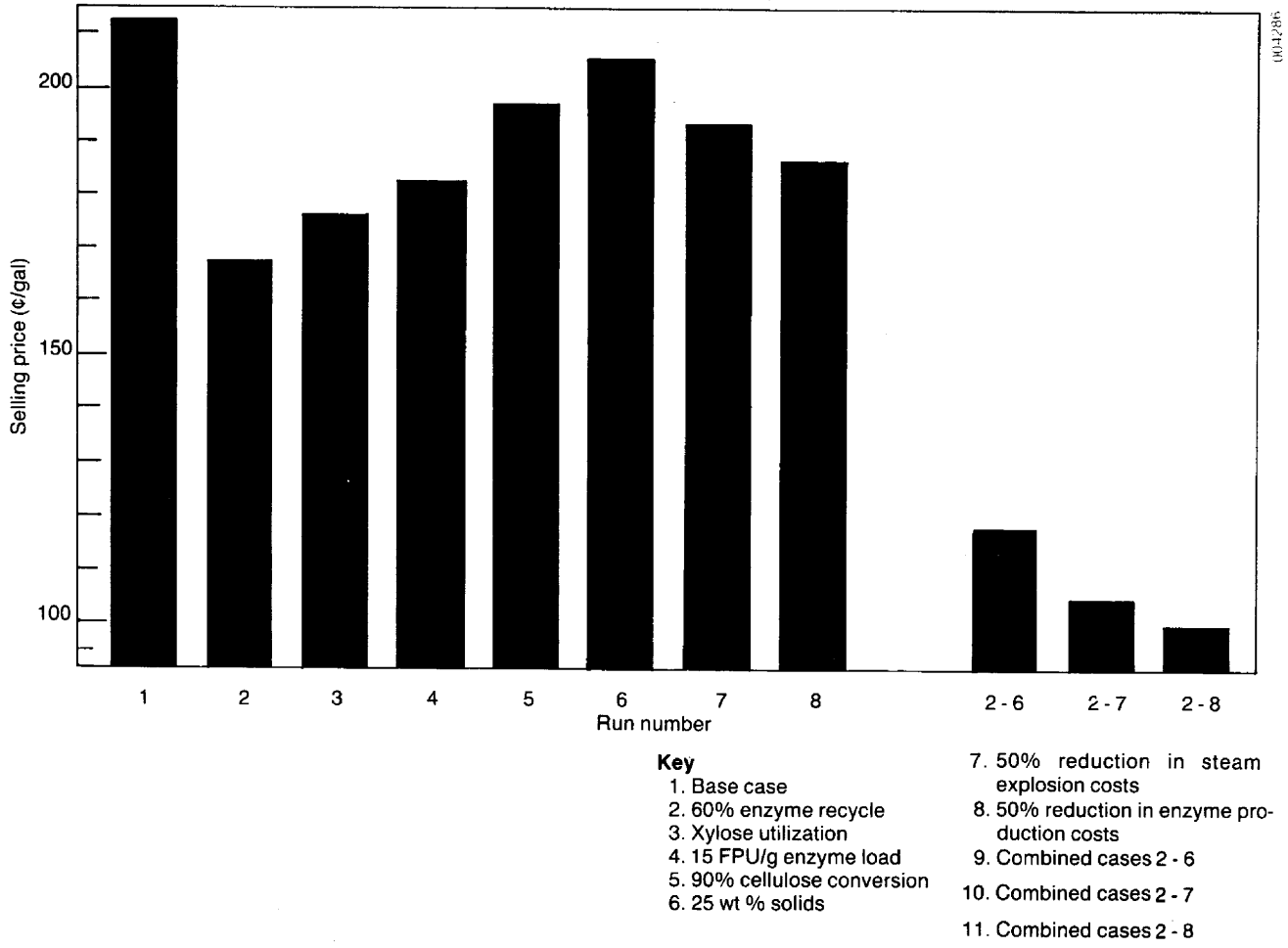


Figure 6-9. Effect of Base-Case Process Improvements on Selling Price of Ethanol

Table 6-5. Contribution to Depreciation of Capital Cost Components (¢/gal)

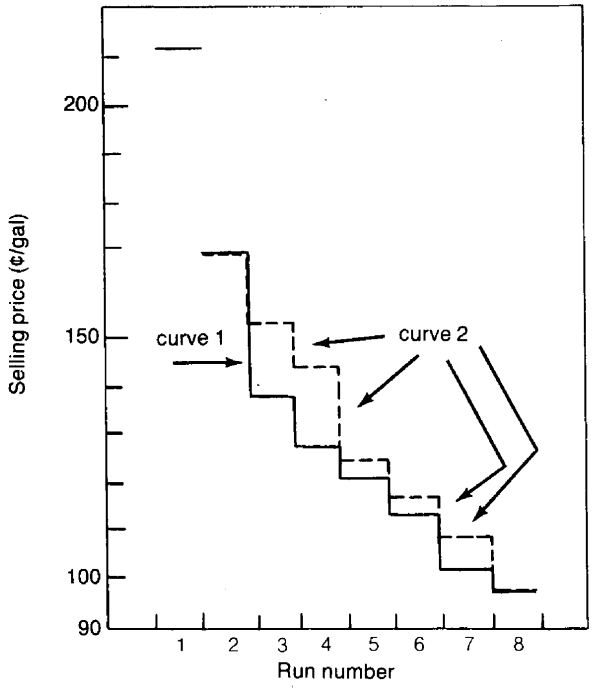
	Steam Explosion	Enzyme Production	Hydrolysis	Fermentation	Purification	Heat Generation	CO_2 Recovery	Total Onsites	Total Offsites	Total Capital Depreciation
1. Base case	18.3	22.1	4.2	2.9	2.6	1.7	3.1	54.9	11.3	66.2
2. 60% enzyme recycle	17.1	8.9	4.1	2.8	2.5	1.6	3.1	40.7	9.6	50.2
3. Xylose utilization	14.6	17.7	3.6	2.7	2.5	1.4	3.1	45.5	10.1	55.6
4. 15 FPU/g enzyme loading	17.6	13.3	4.2	2.8	2.5	1.6	3.1	45.2	10.1	55.3
5. 90% cellulose conversion	16.6	19.9	3.9	2.8	2.5	1.3	3.1	50.1	10.7	60.8
6. 25 wt % solids load	18.5	22.1	3.4	2.7	2.5	1.7	3.1	54.0	10.4	64.4
7. 50% reduction of steam explosion cost	9.7	23.4	4.5	3.1	2.7	1.8	3.1	48.2	9.9	58.1
8. 50% reduction of enzyme production cost	17.7	10.7	4.1	2.8	2.5	1.6	3.1	42.5	10.3	52.8
9. Combined cases 2-6 above	12.1	4.2	2.5	2.3	2.3	1.1	3.1	28.0	7.5	35.5
10. Combined cases 2-7	6.5	4.5	2.7	2.5	2.5	1.2	3.1	23.4	6.6	30.0
11. Combined cases 2-8	6.4	2.2	2.6	2.5	2.4	1.1	3.1	20.9	6.4	27.3

reduction in depreciation for the 7 individual cases (from 66.2¢/gal to 50.2¢/gal, a 24% reduction). The combined cases reduce the capital cost components 46.4%, 54.7%, and 58.8%, respectively, for a cost of 35.5¢, 30.0¢, and 27.3¢/gal--once again, a very significant reduction.

This summary shows that process enhancements that would most reduce ethanol price include enzyme reuse, xylose fermentation, and reduction of enzyme loading. The other parameters reduce the price by 3.5% to 12.6%. No single parameter has been shown to reduce the price to the $1/gal range, but as shown by the three combined cases, this price range can be achieved with a combination of the process improvements.

Figure 6-10 shows the implementation of the seven process improvements in a stepwise fashion. The two curves differ only in the order in which the improvements are implemented. Curve 1 includes xylose fermentation immediately after enzyme recycle, but curve 2 includes xylose fermentation as the last improvement. In both cases, the same final selling price of 99¢/gal is reached.

The cents-per-gallon reduction due to a specific improvement depends on the state of the process prior to its implementation, as seen with the case of xylose fermentation. In curve 1, xylose fermentation causes a 29.0¢/gal reduction ($1.67 to $1.38/gal) but in curve 2, only 14¢/gal ($1.14/gal to 99¢/gal).



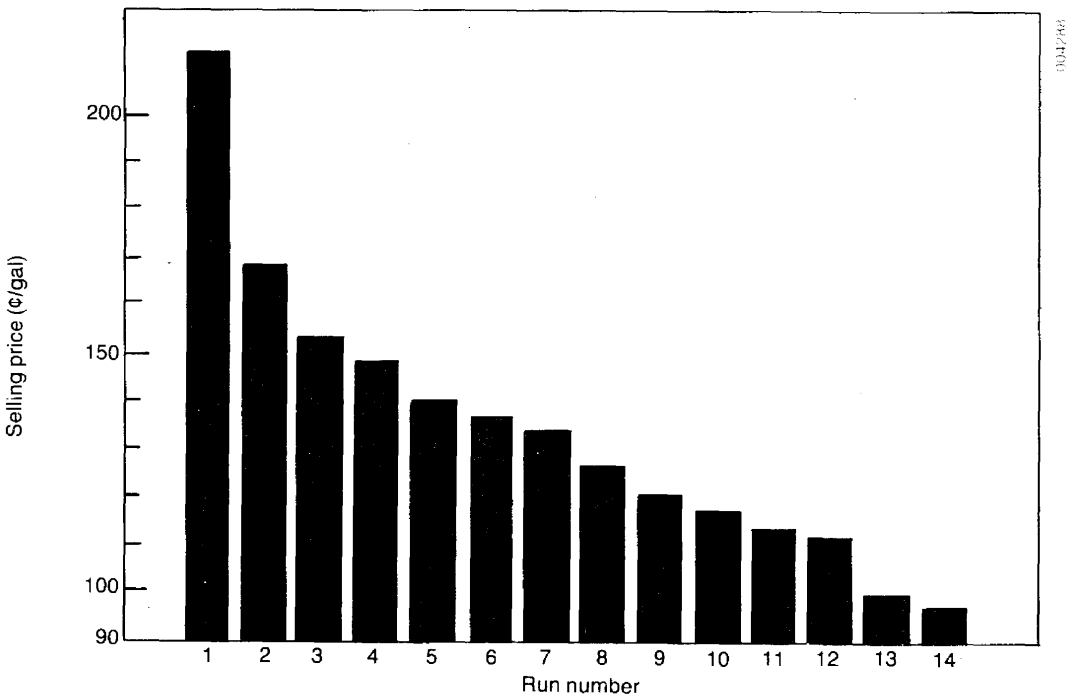
KEY

Run no.	Curve 1	Curve 2
1.	Base case	Base case
2.	Plus enzyme recovery (60%)	Plus enzyme recovery (60%)
3.	Plus xylose utilization	Plus reduction in enzyme load (15 FPU/gram)
4.	Plus reduction in enzyme load (15 FPU/gram)	Plus increased cellulose conversion (90%)
5.	Plus increased cellulose conversion (90%)	Plus increased hydrolysis load (25 wt. %)
6.	Plus increased hydrolysis load (25 wt. %)	Plus 50% reduction in steam explosion costs
7.	Plus 50% reduction in steam explosion costs	Plus 50% reduction in enzyme production capital
8.	Plus 50% reduction in enzyme production	Plus xylose utilization

Figure 6-10. Ethanol Selling Price versus Two Scenarios of Process Improvement Implementations

Figure 6-11 has been included to show that in addition to the major cost reductions provided by enzyme reuse, xylose fermentation, and decreased enzyme load, many smaller reductions can additively cause a significant reduction in price. The simulation shown in this figure also includes reduction of the hydrolysis residence time and reduction of enzyme load to 10 FPU/g, to provide an ethanol price of 96¢/gal.

Figure 6-12 shows the cost contributions, except for by-product credit, for the simulations of curve 1 of Figure 6-10. Costs due to depreciation decrease by the greatest amount. Raw materials costs change significantly with the



Key

Run No.	Improvement
1.	Base-case
2.	60% enzyme reuse
3.	Enzyme load reduction to 15 FPU/g
4.	75% of base-case steam explosion capital cost
5.	50% of base-case steam explosion capital cost
6.	75% of base-case enzyme production capital cost
7.	50% of base-case enzyme production capital cost
8.	25 wt % substrate loading
9.	24 hours hydrolysis residence time
10.	85% cellulose conversion
11.	90% cellulose conversion
12.	75% of base-case steam explosion utilities
13.	Xylose utilization
14.	Enzyme load reduction to 10 FPU/g

Figure 6-11. Ethanol Selling Price versus Process Improvement Implementation

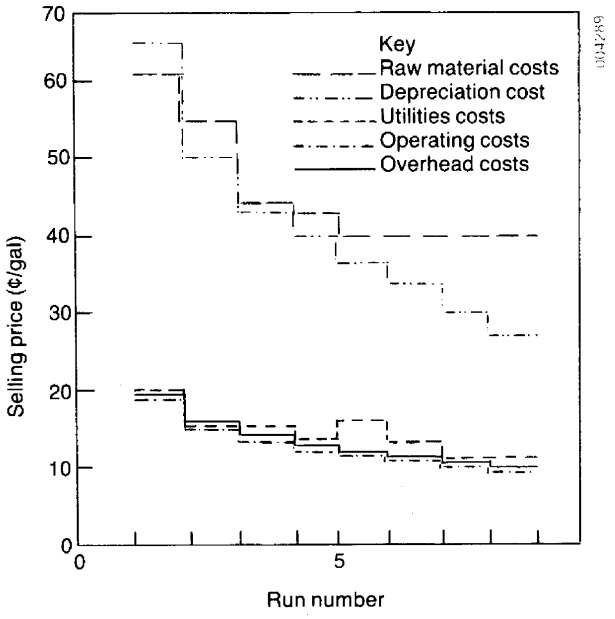


Figure 6-12. Cost Components for Cases of Curve 1, Figure 6-10.

xylose fermentation, enzyme reuse, and increased conversion. The other cost components have a less significant but decreasing trend as each improvement is implemented. Utilities increase as conversion is increased due to a decrease in available residual biomass for onsite steam production.

6.2.4 Selling Price versus Substrate Cost

The base-case process assumes a feedstock of chipped aspen wood at a cost of 75¢/wet lb (50% moisture) or $30/dry ton. Changes in collection radius or changes in the physical size reduction requirements for the process pretreatment step may alter this price. Research may be able to provide a less costly feedstock, for example, through reduction in growth time.

To study the effect of feedstock cost on the selling price of ethanol, simulations were performed in which the feedstock cost was varied from 0¢ to 1.5¢/ wet lb for four process designs. These designs are:

1. Base-case design
2. 60% conversion plus increase in cellulose conversion to 90% plus decrease in enzyme requirement to 15 FPU/g of cellulose
3. Case 2 plus xylose fermentation
4. Case 3 plus 50% reduction in steam explosion costs and enzyme production section capital plus increased hydrolysis solids loading to 25 wt %

Figure 6-13 shows the selling price versus the substrate cost for these four designs. At zero feedstock cost, the base-case selling price is $1.52/gal.

From these simulations, the required feedstock cost to produce a desired selling price can be determined for each of the designs. For example, at a target price of $1.0/gal, the feedstock cost would need to be 0.099, 0.318, and 0.768¢/ wet lb ($4.0, $12.7, and $30.7/dry ton) for designs 2, 3, and 4, respectively.

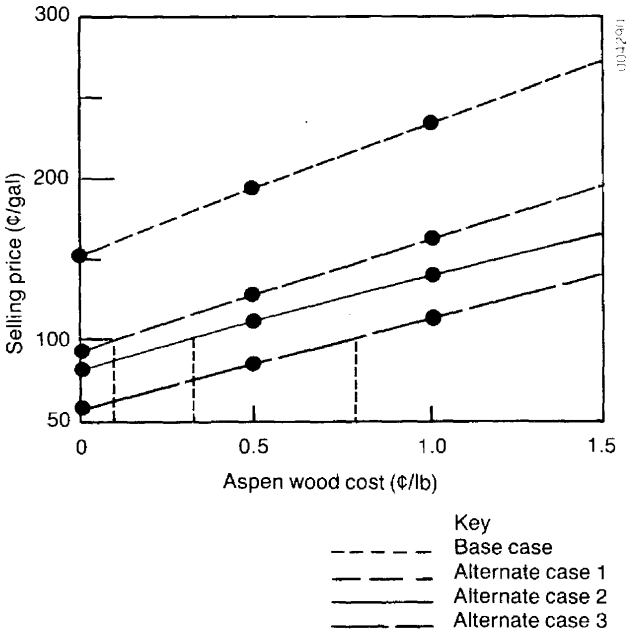


Figure 6-13. Ethanol Selling Price versus Aspen Wood Cost (wet basis). See text for explanation of alternative cases.

SECTION 7.0

DISCUSSION

After careful appraisal of all the assumptions that went into the development of the base-case flowsheet, the current research results that determined these assumptions, and the results of the parametric analysis, it is apparent that future research should follow two major pathways. First, verification on a larger scale of the base-case process verification is needed both to demonstrate that the unit operations of enzyme hydrolysis, enzyme production, and enzyme recycle will perform as anticipated on a larger-than-laboratory scale as well as determine what problems may occur during the operation of the integrated process. Second, basic laboratory research is needed to improve the base-case unit operations in order to improve process economics as demonstrated by the parametric analysis.

7.1 BASE-CASE PROCESS VERIFICATION

The following issues are among those that need to be addressed to verify the base-case economics.

Agitator power requirements: The power requirements for agitation of the cellulosic slurries have been estimated according to procedures by Chemineer Inc. These requirements should be verified for the highly viscous slurries to be encountered in the enzyme hydrolysis, enzyme production, and enzyme recycle sections. In the enzyme production section, due to the metabolic requirements of the fungi, oxygen transfer limitations from the gas to liquid phases may dictate that the power requirements be greater by as much as an order of magnitude (Maiorella 1983).

Materials handling: Rotary wash filters are included in the flowsheet for cellulosic separation in the enzyme production, enzyme hydrolysis, and enzyme recycle sections. It is assumed that 70% moisture could be attained in the filter cake. The filtration properties of cellulose and cellulose plus mycelia slurries may alter the handling requirements and should be evaluated in terms of equipment and performance on a larger scale.

Enzyme production: The ability to produce an enzyme titre of 30 FPU/mL in 13.5 days batch cycle time needs to be verified with the use of the process cellulosic feedstock as carbon source. This performance has been obtained in the laboratory with a fed-batch system using the pure cellulosic carbon source, Solka Floc. Preliminary investigations indicate similar results may be obtained with water-washed steam-exploded substrates (Sciamanna 1983). Fed-batch enzyme production also needs to be evaluated on a larger scale. In particular, due to the high capital cost of equipment in the base-case enzyme production section, studies in fed-batch enzyme production need to be performed using less expensive equipment, i.e., carbon steel, atmospheric vessels with external heat exchangers. If atmospheric equipment is to be used, the adequacy of chemical sterilization will need to be determined.

Steam explosion: Due to proprietary information, the exact steam requirements for steam explosion are not readily apparent. Process development studies will determine the minimum requirements with inclusion of the maximum feasible heat reuse. A recent study reports inhibition of the cellulase enzymes by a toxin present in steam-exploded biomass (Sinitsyn 1982). Water washing of the substrate after steam explosion removed the inhibition. The effects of steam explosion on the hydrolysis of biomass and the subsequent fermentation of soluble sugars should be assessed because it may be necessary to water wash the steam-exploded feedstock prior to hydrolysis as well as enzyme production, and to acclimate the yeast to whatever toxins are present.

Enzyme hydrolysis: The base-case process assumes that 80% conversion of the cellulose can be achieved in 48 hours with an enzyme load of 25 FPU/g of cellulose, at a substrate loading of 20 wt % (with staged-cascaded feed). This needs to be verified on a larger scale due to potential rheological problems encountered with viscous cellulosic slurries and due to potential problems with enzyme inactivation due to product inhibition. Researchers at the University of California at Berkeley laboratories have indicated that cellulase prepared from the RUT-C30 organism contains high levels of the β-glucosidase component (Tangnu 1981). Recent conversations with some Berkeley laboratory staff members have indicated that this β-glucosidase component may have a very short half-life (Sciamanna 1983). In an industrial-scale operation, β-glucosidase enzyme may need to be supplemented from another source. This may be particularly the case when enzyme recycle via adsorption is incorporated, as studies have shown little adsorption of the β-glucosidase component on cellulosics (Orinchowskyj 1982).

Enzyme recovery: Operating conditions for enzyme hydrolysis may vary depending on the level of incorporation of enzyme recycle via adsorption. This is because the various cellulase components adsorb to different extents on cellulose. Fresh enzyme broth would thus have a different component composition than the equivalent broth produced from, for example, 40% fresh makeup enzyme and 60% recycled enzyme via adsorption (based upon filter paper activity). This may cause a significant variation in hydrolysis results. Larger scale simulations of the integrated process, with and without enzyme recycle, should be performed to determine any changes in operational performance.

7.2 BASIC LABORATORY RESEARCH

The following are recommendations for further research to improve the base-case process economics, based on conclusions drawn from the parametric analysis.

Of primary importance is the need for a correlation of cellulose conversion versus pretreatment, feedstock, and hydrolysis operating conditions. Many attempts to model or correlate the enzymatic hydrolysis reaction can be found in the literature, but to date none of the models considered all the major parameters or they have been derived from experiments performed at operating conditions too far removed from that required for an economical process. A simple correlation would facilitate more useful process modeling by enabling a prediction of conversion efficiencies, and thus process economics, for

variations in the base-case process. A more detailed mechanistic model that would describe the microscopic events of enzyme hydrolysis could, in addition, define research pathways toward the goal of process improvement.

Research directed toward reduction in enzyme production costs would be of major benefit to process economics. Fundamental research on strain improvement of Trichoderma reesei should continue to allow production of cellulase in a less costly manner. Alternate fungal and bacterial strains should also be considered, because T. reesei may not be the optimal cellulase source. Process research and engineering of the enzyme production section may lead to a decrease in the high capital and operating costs.

In addition to further process studies on the recycle of cellulase via adsorption onto incoming cellulosics, other methods of enzyme recycle should be researched that may allow a greater percentage recovery and an equal recovery for each of the cellulase components. Ultrafiltration equipment is continually decreasing in price and could provide an alternate means of cellulase recycle. One study indicates that enzyme adsorbed onto residual solids may be recovered with washing techniques (Sinitsyn 1983).

A major improvement would be the development of organisms that have the ability to ferment five-carbon as well as six-carbon sugars to ethanol. In this manner, substrate utilization would be increased and the plant capacity of the front end (steam explosion through hydrolysis) would be decreased due to the production of additional ethanol via fermentation of the xylose.

Further fundamental and process research on pretreatment is required. Pretreatments that allow high conversion efficiencies in enzymatic hydrolysis have been identified, but processing costs are high. Research should be directed toward the development of new, cost-effective pretreatments as well as toward cost reduction of current methods of pretreatment.

Lignin has the potential to be a valuable by-product. Research is required to identify methods of lignin recovery and better define its uses. Removal of lignins prior to hydrolysis may have the following advantages: (a) separation of the potentially valuable lignin by-product from cellulose; (b) removal of the inert lignin from the hydrolysis slurry, which at high substrate loadings would improve hydrolysis agitation; and (c) removal of lignins as a potential site for cellulase adsorption.

Removal of lignins prior to hydrolysis may have another benefit. An effective pretreatment such as steam explosion may render the cellulose totally accessible to enzymatic hydrolysis within a reasonable reaction time. Therefore, limits on conversion percentage may be due to product inhibition rather than slow reaction rates caused by inaccessible regions of cellulose. If this were the case and the lignins were removed, cellulose conversion may be enhanced with some form of cellulose recycle. Cellulose recycle may also aid in enzyme recycle by allowing the reuse of the enzyme adsorbed to the recycled cellulose.

One study indicated that higher levels of effective cellulase activity were obtained when whole culture broth from enzyme production was used rather than culture filtrate alone (University of Arkansas 1982). Most studies that form

the basis for the assumptions of the base-case process presented here were performed with culture filtrate. Use of whole culture broth should be studied because it may enhance the levels of cellulase activity obtained per batch as well as simplify the enzyme production section by removal of the filtration-wash step.

Finally, research should continue in alternative enzyme hydrolysis processes. Due to the combining of processing steps, the simultaneous saccharification and fermentation (SSF) process and the direct conversion process have great potential toward reduction of costs and process complexity. Continuous fermentation of sugars to ethanol as they are formed also would relieve inhibition of cellulase by glucose.

SECTION 8.0

CONCLUSIONS

A base-case selling price of ethanol of $2.13/gal has been demonstrated by the simulation model for a 50 x 10^6 gal/yr plant. This price is not competitive with the current market price for ethanol production from ethylene or starch and sugar crops, or via a proposed acid hydrolysis process (Wright 1982). However, the parametric analysis of this study indicates that significant reductions to this price can be attained through process research and engineering.

Due to the degree of uncertainty of the process performance for the proposed operating conditions at an industrial scale (as described in Section 7.0), uncertainty in the feedstock costs, and lack of detail in the simulation model for the back end process (of the fermentation, purification, and waste treatment sections), the actual costs per gallon of ethanol as determined by this simulation model should be considered only as approximations and as a method for identifying research areas with significant impact on costs. However, the results may be comparable with those of the acid hydrolysis process proposed by Wright (1982), because the simulation models were similar with respect to the back end of the plant and economic assumptions. In the referenced report, the base-case cost for the high-temperature acid hydrolysis process of $2.15/gal was reduced to $1.45/gal or as low as $1.10/gal when xylose fermentation was included. In both cases, aspen wood cost $20/wet ton, and furfural was not recovered for by-product credit. The parametric analysis of the current study of an enzyme hydrolysis process showed a potential decrease in ethanol cost to as low as 99¢/gal with a combination of process improvements (Figure 6-10) at a feedstock cost of $15/wet ton. For comparison for aspen wood priced at $20/wet ton (1¢/wet lb) these improvements produce a decrease in price to as low as $1.12/gal (see Figure 6-13).

The most significant cost reductions can be achieved through incorporation of the enzyme recycle section for 60% enzyme recovery and fermentation of xylose. Combination of these two improvements alone reduces the base-case price to $1.40/gal (curve 1 of Figure 6-10). Reduction in enzyme loading per gram of cellulose will also cause a significant price reduction if the cellulose conversion is unaffected and the required hydrolysis residence time is not greatly increased. To a lesser but still significant degree, price reduction can be achieved with an increase in hydrolysis solids loading, an increase in cellulose conversion, and a decrease in enzyme production and pretreatment capital and operating cost.

Ethanol price has the potential for further reduction by improvements not included in the derivation of Figure 6-10. These include additional by-product credits (lignins and furfurals formed during steam explosion), and improvements in cellulase-producing organisms that may provide higher enzyme titres, shorten batch production times, or remove the requirements for a cellulose carbon source.

In conclusion, the parametric analysis of this study points out key areas of research that will significantly improve the base-case process economics.

Careful scrutiny of the simulation model and the process flowsheet also point out key areas of research still to be performed that would verify the base-case process performance on an industrial scale. Most important is the demonstration of this analysis that this particular enzymatic hydrolysis process may allow ethanol production at a price competitive with current ethanol production technologies and with the acid hydrolysis cellulose-to-ethanol process.

SECTION 9.0

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APPENDIX A

ALTERNATIVE ENZYMATIC HYDROLYSIS PROCESSES

It is beyond the scope of this report to include a parametric analysis and evaluation of alternative enzymatic cellulose-to-ethanol processes. Many of these alternatives are just in the early stages of laboratory research. However, they have the potential to provide significant cost reduction, so a brief discussion of the flowsheets is provided below.

Enzymatic hydrolysis processes can be grouped into three classes. The first class, which was evaluated in this report, is based on separate hydrolysis and fermentation stages. The next class has often been referred to as simultaneous saccharification and fermentation (SSF) and combines hydrolysis and fermentation in one vessel. This is accomplished by introduction of a glucose-fermenting organism into the hydrolysis vessel along with the biomass feed and cellulase. The final class, referred to as direct conversion processes, combines all three process steps of enzyme production, hydrolysis, and fermentation in one vessel.

SSF processes have the advantage of reducing equipment costs due to the combination of two processing steps. The required degree of asepsis in the hydrolysis reaction is reduced due to the presence of ethanol and anaerobic conditions of yeast fermentation. Hydrolysis rates, sugar yields, and ethanol yields are potentially higher due to continuous removal of the glucose and cellobiose inhibition of the cellulase enzymes. A major disadvantage of the SSF concept is that the operating conditions must be a compromise between those for cellulase enzymes and those for the fermenting organisms. For example, fungal cellulases have a temperature optimum between 45° and 50°C, but Saccharomyces cerevisiae optimally operates at 30°C.

An SSF process was developed by Gulf Oil and Chemical Company and later transferred to the University of Arkansas (Emert 1980). The basic steps of this process are shown in Figure A-1. A mutant strain of Trichoderma reesei is used as the cellulase source. The enzyme is added to the hydrolysis vessels as whole culture broth, with no concentration or filtration, along with the pretreated biomass. Yeast (Saccharomyces cerevisiae and Candida brassicae are the preferred yeasts) is added either as a cake or recycled as a cream.

Direct conversion processes further simplify process design by combining the enzyme production step with hydrolysis and fermentation. This is achieved with use of an organism or compatible mixture of organisms that will grow on cellulose and convert it to sugars and ethanol. Current problems associated with direct conversion are that growth and reaction rates are relatively low, and significant amounts of by-products may be produced at the expense of ethanol.

Figure A-2 shows the Battelle design of the MIT direct conversion process. A mixed culture of Clostridium thermocellum and Clostridium thermosaccharolyticum is used. C. thermocellum cellulase hydrolyzes both cellulose to glucose and hemicellulose to xylose. This organism also ferments glucose to ethanol but cannot utilize xylose. C. thermosaccharolyticum is added to

ferment xylose and is environmentally and biologically compatible with C. thermocellum.

More detailed descriptions can be found in the literature on direct conversion processes developed by MIT (Jenkins 1979) and GE/CRD (Brooks 1979).

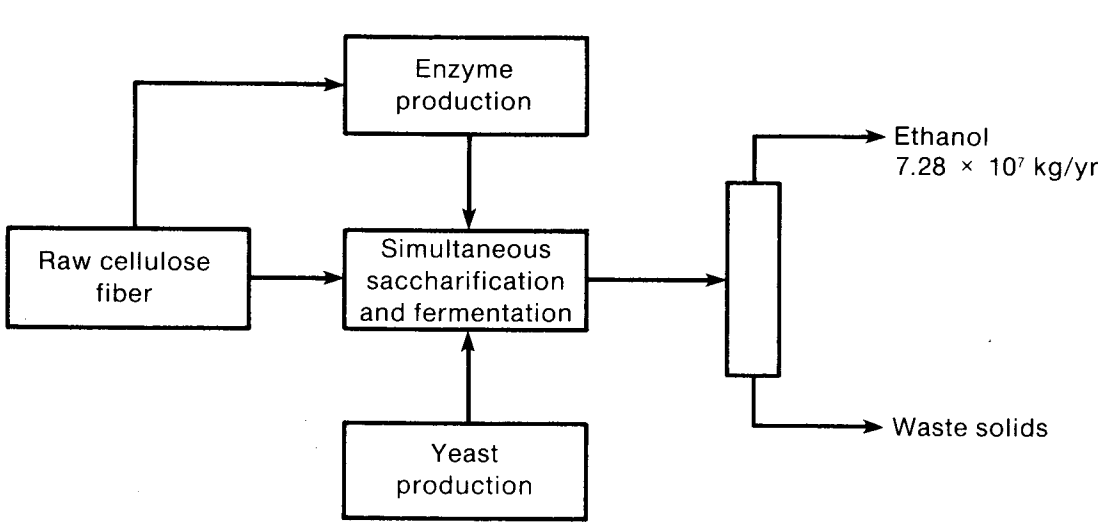

Figure A-1. Gulf/University of Arkansas Process
Source: Wilke 1980

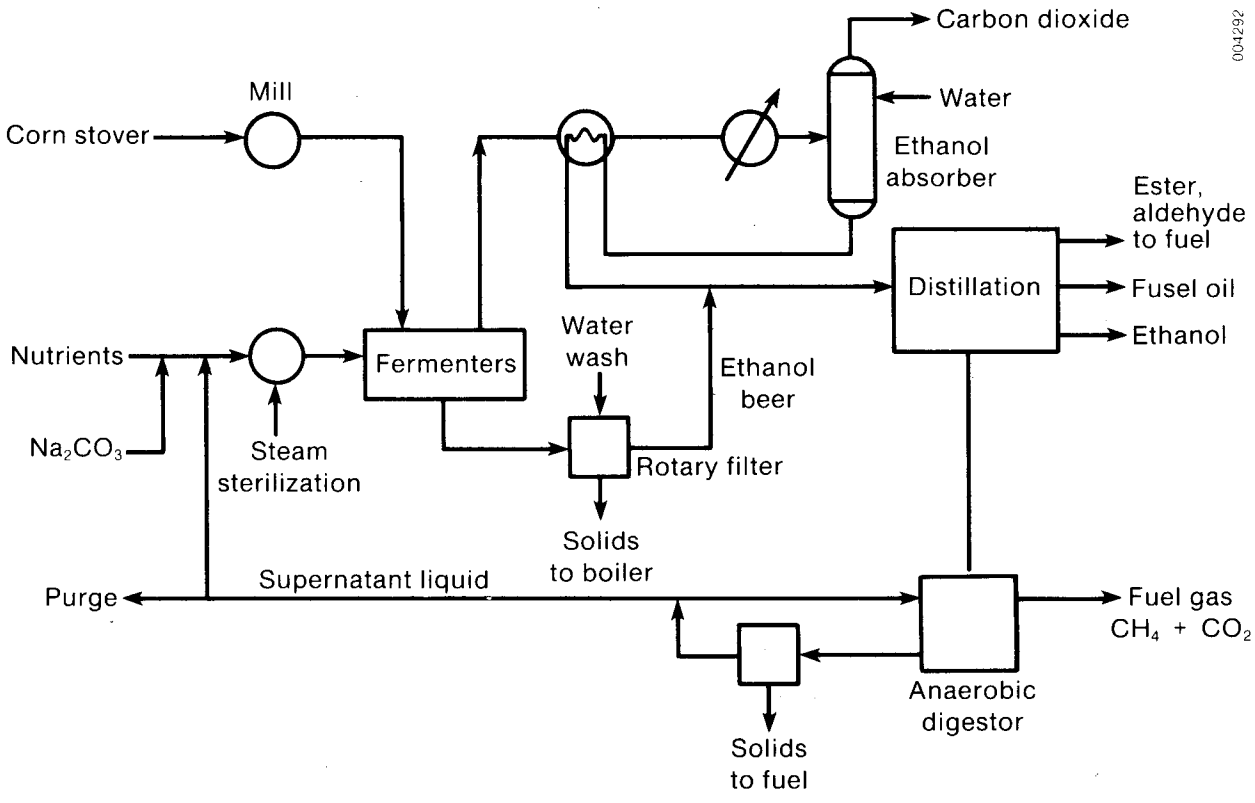

Figure A-2. Battelle Design of MIT Process
Source: Wilke 1980

APPENDIX B

ENZYMATIC HYDROLYSIS EXAMPLES

To provide a background for the discussions and assumptions of this study, a few examples of enzymatic hydrolysis are presented here. Many mechanistic models of the action of cellulases on cellulose have been proposed, and an excellent review is provided by Fan (1980a, 1980b). However, none of these models encompass all the factors that may affect conversion. Some of the more comprehensive models may not be applicable to the conditions of the base-case process of this study. As an example, Chem Systems developed a complex empirical formula to describe the percentage of conversion for varying operating conditions based on laboratory data using Dartmouth acid-pretreated biomass and hydrolysis solids loadings under 10 wt %. This formula may not apply to the current base-case process that employs steam explosion as pretreatment and hydrolysis loading of 20 wt %.

Figure B-1 is an example of a typical hydrolysis of steam-exploded corn stover using T. reesei cellulase. Glucose formation is extremely rapid in the initial stages of reaction due to the ease of hydrolysis of the amorphous cellulose. Glucose production steadily decreases as the remaining cellulose becomes less accessible (more crystalline or shielded by lignin). At high reaction times, glucose formation may reach a maximum at less than 100% conversion due to deactivation of the enzymes (thermal instability or product inhibition) or due to inaccessibility of the remaining cellulose.

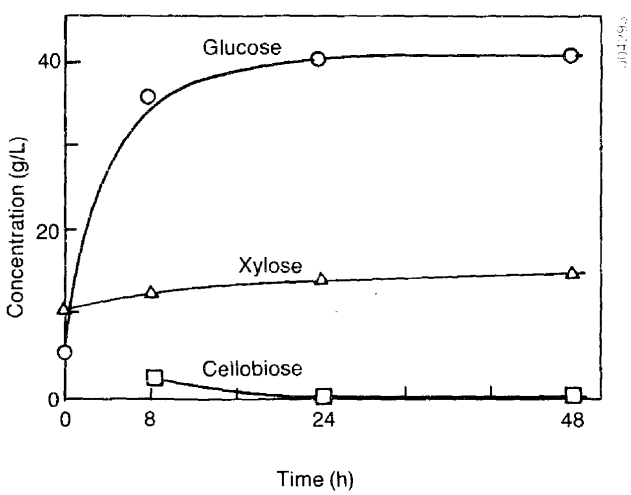

Figure B-1. Example of Enzyme Hydrolysis at High Enzyme Loading (63 FPU/g solids). Substrate is steam-exploded corn stover. Source: Perez 1981

At high specific enzyme activity (63 FPU/g biomass or approximately 150 FPU/g cellulose in Figure B-1), enough β-glucosidase is present in the cellulase system of T. reesei to convert the cellobiose to glucose at long reaction times. Cellobiose accumulates initially during the period of high cellulose hydrolysis rate. Figure B-2 shows an example of the hydrolysis of steam-exploded corn stover with low enzyme load (10 FPU/g biomass, or approximately 25 FPU/g cellulose). Although cellobiose is consumed continuously after the initial few hours, a significant amount remains after 48 hours. Glucose production proceeds more slowly and does not appear to have reached the maximum after 48 hours.

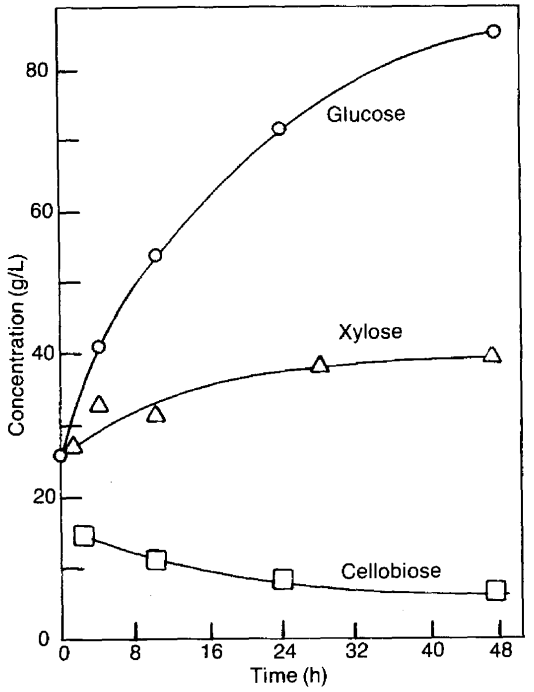

Figure B-2. Hydrolysis of Steam-Exploded Corn Stover at Low Specific Cellulase Activity
Source: Perez 1981

Figure B-3 shows the relationship between the percentage of cellulose conversion at 48 hours and the enzyme load using steam-exploded corn stover. Maximum conversion of 82% occurs at approximately 30-40 FPU/g. Additional enzyme will not increase the 48-hour conversion but would perhaps allow a reduction in residence time. Increased residence time may shift the maximum to lower enzyme loads.

The interactions between enzyme load, substrate load, and 48-hour conversion are illustrated for acid-treated corn stover in Figure B-4. As substrate load is increased, the 48-hour conversion decreases, possibly as a result of product inhibition at the higher substrate loadings. As the enzyme load is decreased, this trend becomes more significant. This can be explained by product inhibition due to cellobiose accumulation. At lower

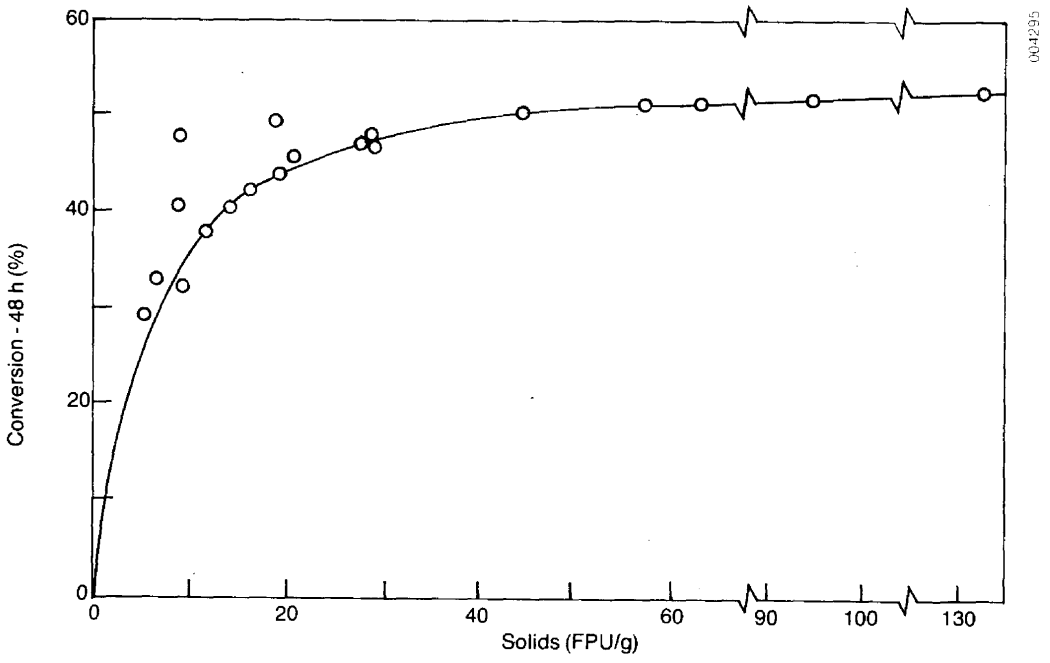

Figure B-3. Glucose Conversion versus Specific Activity. Acid-treated corn stover (Calif.), cellulase from RUT C30 (11/80)
Source: Perez 1981

enzyme loadings, more cellobiose will accumulate due to insufficient β-glucosidase activity.

Figure B-4 shows the relationship between hydrolysis residence time, substrate load, and enzyme load. At high enzyme load (circles), maximum conversions may be reached at shorter residence times as substrate load is decreased. This is illustrated by the fact that at 5 wt % substrate and 7 FPU/g enzyme, there is no conversion increase between 24 and 48-hour residence time. As substrate load increases, more time is required to reach the maximum conversion. As the enzyme load is decreased, this trend becomes more significant.

An example of the effect of pretreatment can be seen in Figure B-5. The source of this figure did not indicate the percentage of conversion at 24 hours, and the exact cellulose content of the steam-exploded and acid-treated corn stover was unknown. However, at 24 hours the steam-exploded corn stover produced 1.8 times the glucose of the acid-pretreated corn stover.

The parametric analysis in Section 6.0 of this study indicated a significant reduction in cost of ethanol production when 60% of the enzyme is recycled via countercurrent adsorption. The hypothesis that at least 60% of the filter paper activity can be recycled in this fashion is based on laboratory studies carried out at the University of California at Berkeley (Orinchowskyj 1982).

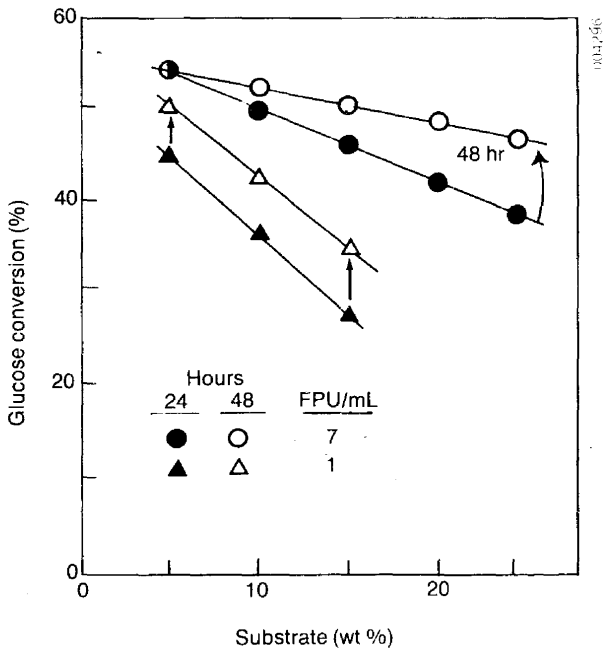

Figure B-4. Example of Interaction of Substrate Loading (FPU/mL), Reaction Time, and Enzyme Loading on Hydrolysis of Acid-Treated Corn Stover
Source: Perez 1981

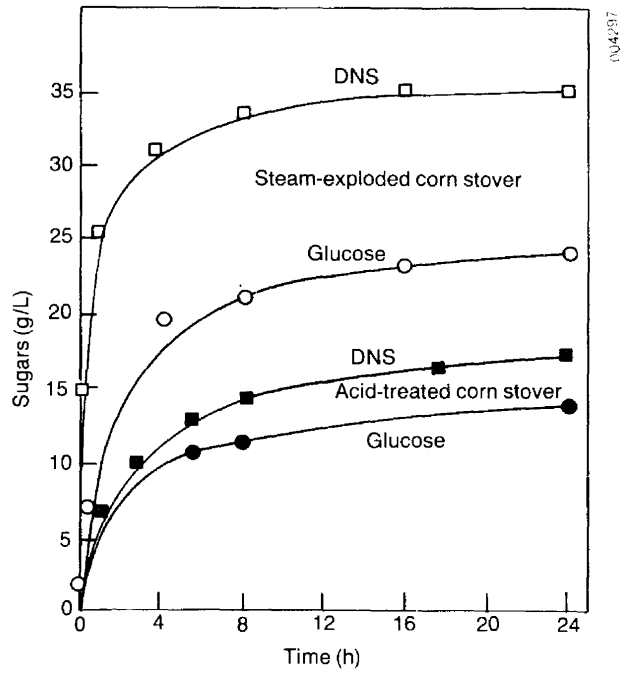

Figure B-5. Effect of Pretreatment on Hydrolysis of Corn Stover. Data for acid-treated corn stover from LBL report LBL-14223.
Source: Orinchowskyj 1982

Figure B-6 shows an example of the disappearance of cellulase activity from solution when contacted with a 5 wt % solution of steam-exploded corn stover. The majority of adsorption occurs within the first two minutes of contact. The relative amounts of adsorption of the various cellulase components are consistent with their affinity for the substrate; β-glucosidase, which acts on the soluble cellobiose, adsorbs the least whereas the C_1 activity, which attacks the crystalline cellulose, adsorbs the most. Filter paper activity (FPA) is an estimation of the combined cellulase activities, and the amount of adsorption is between that of the C_x and C_1 activities.

At low cellulose conversions, the majority of this activity remains adsorbed onto the solids and enzyme recycle first requires desorption. Washing with water was shown to desorb the C_x activity but not C_1 (Orinchowskyj 1982). Desorption can be achieved with addition of urea, but an analysis of such a process shows unfavorable economics due to the cost of urea (Wilke 1982).

At high cellulose conversions, however, such as those achieved with steam-exploded biomass, a major portion of the enzyme activities is released. Figure B-7 indicates that after 24 hours approximately 80% of the original FPA is present in solution. Theoretically, this activity can be readsorbed onto fresh hydrolysis biomass feed and, in this manner, recycled back to the hydrolysis vessel.

Langmuir isotherm constants can be determined from adsorption data such as those shown in Figure B-6. These constants are specific for the particular enzyme preparation, substrate, and pretreatment, and they describe the adsorption of the enzyme according to the equation:

$$\frac{E_{ads}}{E_{max}} = \frac{KE}{(1 + K)} ,$$

where

E_{max} = maximum level of enzyme adsorption (units/g)

E_{ads} = enzyme adsorbed onto solids at equilibrium (units/g)

E = enzyme activity in solutuion (units/mL)

K = constant (mL/units).

Using the langmuir isotherm constants for steam-exploded corn stover of K = 0.329 mL/FPU and E_{max} = 133 FPU/g, and assuming a conservative estimate that 65% of the FPA available is in solution after hydrolysis, simulations of a two-stage countercurrent adsorption reactor conducted at SERI indicates that as much as 97% of this soluble FPA may be readsorbed onto the fresh hydrolysis feed. Thus, in excess of 60% of the original FPA can be recycled and reused.

At large percentages of enzyme recycle via adsorption, the steady-state cellulase composition will differ from that of native cellulase due to the difference in affinity of the various components for the solid substrate, as shown

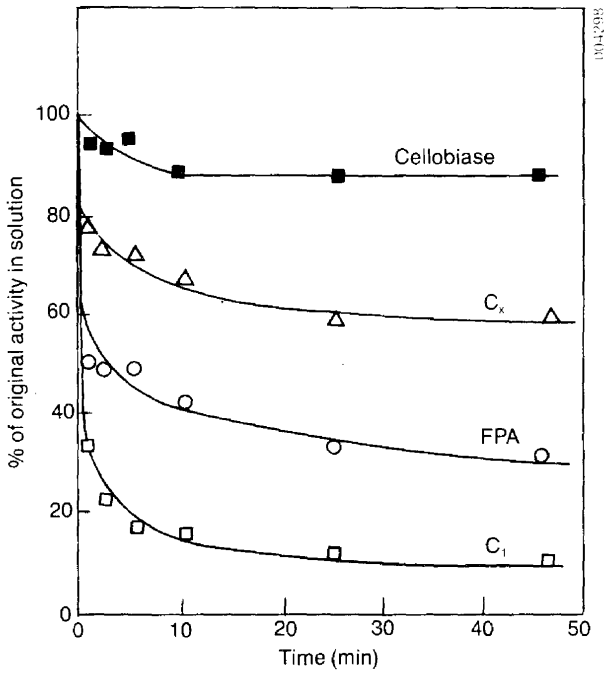

Figure B-6. Adsorption of Cellulase Enzyme Activities onto 5 wt % Steam-Exploded Corn Stover at 15°C and Enzyme Load of 25 FPU/g Solids

in Figure B-6. For example, a mixture of 60% recycled enzyme and 40% make-up enzyme (based on FPA) may contain as little as 40% of the original β-glucosidase component. This may have a pronounced effect on the hydrolysis kinetics due to the inhibition caused by the accumulation of cellobiose. Figure B-8 shows the accumulation of cellobiose at 48-hour hydrolysis time versus the β-glucosidase loading in international units (IU) per gram. Figure B-9 shows how the cellulose conversion at 24 hours is decreased as the ratio of β-glucosidase to cellulase is decreased for a range of enzyme-to-substrate loadings. In order to maintain a sufficient level of β-glucosidase in the hydrolysis reactors, either a certain minimal amount of fresh cellulase with sufficient β-gluosidase activity must be supplied, or β-glucosidase must be supplemented from another source. In the study at the

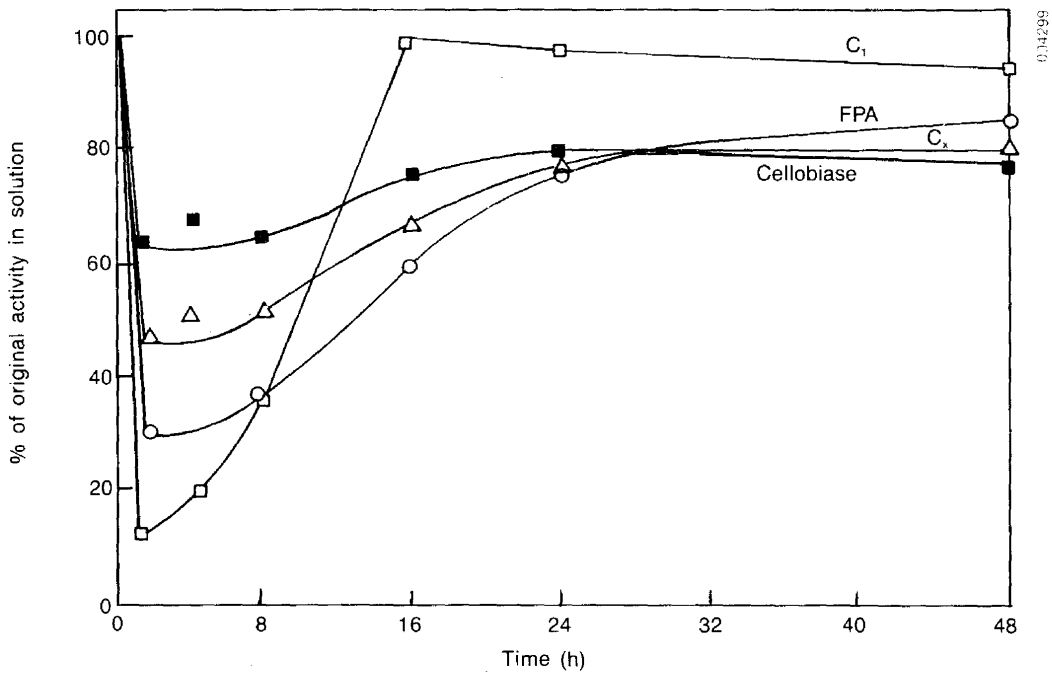

Figure B-7. Adsorption and Desorption of Cellulase Enzymes during Hydrolysis of Steam-Exploded Corn Stover
Source: Orinchowskyj 1982.

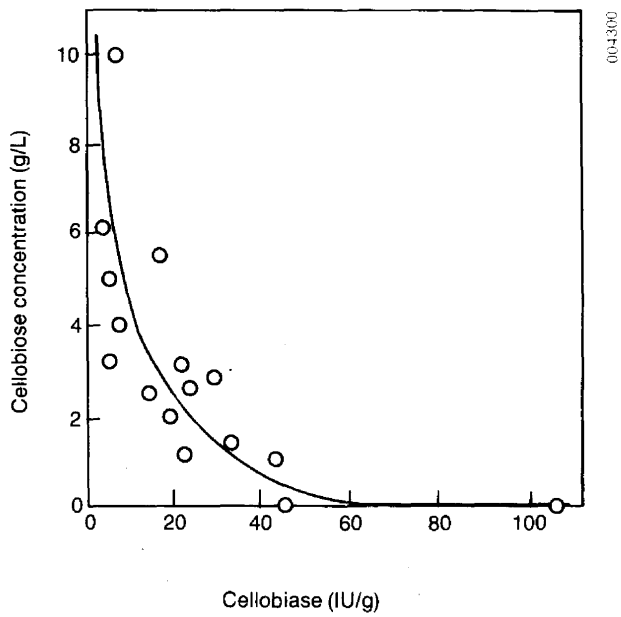

Figure B-8. Cellobiose Concentration Observed after 48 hours of Hydrolysis versus Specific Cellobiase (β-glucosidase) Activity
Source: Perez 1981

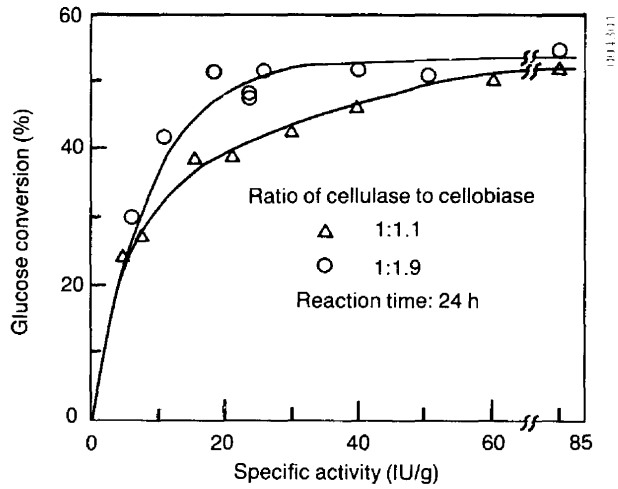

Figure B-9. Glucose Production versus Specific Cellulase Activity for Different Ratios of Cellobiase (β-Glucosidase) to Cellulase
Source: Perez 1981

University of California at Berkeley, bench-scale simulation of a two-stage countercurrent adsorption reactor and hydrolysis vessel indicated that a steady-state level of β-glucosidase could be obtained if make-up enzyme from RUT C30 was at least 35%, based on FPA.

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A process design is presented for the production of 50 million gallons per year of fuel ethanol from cellulose through enzymatic hydrolysis. The base-case selling price for ethanol of $2.13/gal is estimated for this process utilizing steam explosion pretreatment of aspen wood feedstock, fed-batch enzyme production with Trichoderma reesei, separate hydrolysis and fermentation, and vacuum distillation. A parametric sensitivity analysis is performed with a computer simulation model to study the influence of selling price on key process parameters, which include enzyme recycle, enzyme loading, residence time, cellulose conversion, xylose utilization, and feedstock use.

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